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ON THE QUALITY OF BREAD FROM WHEATS SUPPLIED WITH NITROGEN AT DIFFERENT STAGES OF GROWTH

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That the protein content of wheat may vary with the supply of available nitrogen to the plants, has been shown by Davidson and Le Clerc (1917); Gericke (1920); and Moertlbauer (1911). The method employed consisted of supplying a soluble salt of nitrogen to the plants at various stages of their development. The writer has tried this method on fifteen varieties of wheat, and with all spring varieties obtained products that defined a range of percentages of protein in the grain characterized as low to very high. The magnitude of the range varied with each variety but in all cases, whether the wheat was a spring or a winter variety, an application of nitrogen at certain times produced grain as high in protein as was known to be possible for that variety. It thus appears, in the light of the physiological principle involved, that any variety of wheat known to vary in percentage of protein can be made to produce markedly high protein grain by the simple method of supplying nitrogen to the plants at the proper growth stage. This holds regardless of any climatic factor to which the plants may be exposed. However, climatic factors influence conditions that determine the proper growth stage, so that the method needs to be modified to meet any given condition.

The percentage of nitrogen in the grain of any variety may be considered as being the result of several factors. Of these, the following are the most important: (1) the genetic character of the variety and (2) the amount of nitrogen the plant has absorbed that is available for grain. The genetic character of the plant determines the upper and lower limits of the range of potential variation in the protein content of the grain. The amount of nitrogen the plant has absorbed for use in the grain determines what

the actual percentage of nitrogen within the range will be. It is necessary to emphasize the amount of nitrogen absorbed by the plant available for *grain* in contra-distinction from that absorbed by and available to the plant as a whole. It is in this category pertaining to the differentiation of the nitrogen available for and to the grain from that available to the plant as a whole, as effected by the apportionment of the supply of available nitrogen in the soil during the growth of the plants, that soil and climate give rise to variations in the protein content of wheat. Climate affects the biological activities in the soil and obviously influences the supply and distribution of nitrogen to the plants. This is the means by which it is responsible for seasonal and regional differences in the protein content of wheat. That it likewise affects the quality of the flour in addition to the protein content of the grain, is a point to be developed in this paper.

The quality of the flour of the standard bread wheats usually expressed by the term "strength" is related in a general way to the protein content of the grain—the higher the protein in the grain, the better and stronger the flour. However, there are exceptions to the rule. This is usually referred to as differences in the quality of the gluten, a term used by millers and bakers to express the relative excellence of the protein in wheat. So far, cereal chemistry has failed to identify or associate this "quality" with definite chemical or physical properties of either grain or flour. This leaves the actual baking test as the only infallible means of determining the relative merits of any wheat for flour purposes.[1]

The regional and seasonal character of the exception to the general rule on the relation of the quality of the flour to the percentage of protein in the grain, suggests that small differences in the growth habits of the plants from that of a given standard type of response, may contain certain features that are related to variations in the quality of the flour as determined by an actual baking test. If the quality of the bread from wheats of the same variety, grown in different sections or in different years, but having the same percentage of protein, varies, the wheats conceivably had also varied in growth habits as a result of differences in conditions of culture, or because of influence of soil and climate. As already stated, the effects of the peculiarity of any soil or climate in its

1 This statement may be criticized as being too dogmatic and not in accord with the milling practices in certain localities. While it is true that flours produced from wheats grown in certain sections vary in strength with the protein content of the grain, yet the fact remains that flour produced from wheat grown in other sections may or may not be related correspondingly to variation in the protein content of the grain.

relation as cause for variation in the protein content of the grain necessarily must operate through the nitrogen factor. It was thought possible that by supplying nitrogen to the plants one may, in laboratory experiments, simulate in a measure the aggregate effect of diverse climates. It was also thought probable that by employing an array of varieties whereby a fairly large range of reactions would obtain, certain clues would develop that would enable one to determine what characteristics of the plant are associated with the quality of flour apart from the protein content of the grain.

Six varieties of wheat were planted side by side and each received the same treatment. The varieties comprised a range from early to late spring wheat. They likewise constituted a fairly wide range in respect to a very important property of wheat, namely, the capacity of a variety to change in protein content. This has been discussed by the author (1925) in another paper. Planting was made in the open field. The grain was sown in rows ten inches apart. At the time of planting, a portion of the plot of each variety received an application of sodium nitrate equal to the rate of 100 pounds of nitrogen per acre. Other portions of the plots received similar applications of sodium nitrate at subsequent growth periods, as is indicated by Table I. Save for irrigation by means of over-head sprinkling, the cultural conditions were those common to field practice. The wheat was harvested when ripe. The data are given in Table I.

TABLE I

DATE OF HARVEST OF WHEATS SUPPLIED WITH NITROGEN AT DIFFERENT STAGES OF DEVELOPMENT

	Date of application of nitrogen	Bunyip	Early Baart	Hard Federation	Jenkins Club	Pusa	Sonora
No. 1	March 15—two weeks after planting, plants were about two inches above ground.	July 4	July 6	July 3	Aug. 5	July 1	July 6
No. 2	April 15	July 6	July 6	July 3	Aug. 5	July 1	Ju ly 6
No. 3	May 15	July 9	July 6	July 14	Aug. 5	July 1	July 16
No. 4	No nitrogen	July 6	July 6	July 14	Aug. 5	July 1	Jul y 1 6
No. 5	No nitrogen	July 6	July 6	July 14	Aug. 5	July 1	July 16

The chief point to be noted in this table is the effect of the treatments on the maturity of the various samples of each variety. The treatment did not alter the date of maturity of Early Baart, Jenkins Club, and Pusa. However, Bunyip, Hard Federation, and Sonora did not ripen together, hence it is inferred that the treatment altered the date of maturity of some of the samples from what would be considered as normal.

TABLE II

DATA ON CHEMICAL AND BAKING PROPERTIES OF WHEATS SUPPLIED WITH NITROGEN AT DIFFERENT STAGES OF GROWTH

Variety and time of fertilizing	Total protein per cent	Wheat moisture, per cent	Absorption, per cent	Gluten			Wt. of loaf, gm.	Loaves per barrel	Vol. of loaf, cc.	Texture of bread in order of excellence
				Per cent	Color	Quality				
BUNYIP										
No. 1, 15 days after planting	12.30	12.8	59	30.7	Yellow	Good	498	295	2060	3
No. 2, 45 days after planting	13.90	12.8	59	34.6	Yellow	Good	500	295	2100	2
No. 3, 75 days after planting	15.10	13.0	59	37.7	Grayish	Soft	497	295	2125	1
No. 4, untreated but adjacent to No. 3	12.40	12.6	59	31.0	Grayish	Soft	505	295	2025	4
No. 5, untreated	12.35	12.6	59	30.8	Grayish	Very soft	500	295	2025	5
EARLY BAART										
No. 1, 15 days after planting	11.80	12.8	57	29.5	Yellowish	Soft	493	291	2150	1
No. 2, 45 days after planting	13.10	12.8	57	32.7	Yellowish	Soft	495	291	1950	4
No. 3, 75 days after planting	13.80	12.7	57	34.5	Yellowish	Soft	495	291	2000	5
No. 4, untreated but adjacent to No. 3	12.30	12.6	57	30.7	Yellowish	Soft	492	291	2070	2
No. 5, untreated	12.10	13.0	57	30.2	Yellowish	Soft	496	291	2080	3
HARD FEDERATION										
No. 1, 15 days after planting	11.75	12.2	58	29.3	Yellow	Good	500	293	1900	3
No. 2, 45 days after planting	13.00	12.6	58	32.5	Yellow	Good	502	293	2080	1
No. 3, 75 days after planting	14.15	12.2	58	35.3	Yellow	Good	505	293	2120	2
Nos. 4 and 5, untreated but adjacent to No. 3	10.60	12.5	60	26.5	Yellowish	Good	508	296	2025	4

TABLE II—*Continued*

DATA ON CHEMICAL AND BAKING PROPERTIES OF WHEATS SUPPLIED WITH NITROGEN AT DIFFERENT STAGES OF GROWTH

Variety and time of fertilizing	Total protein per cent	Wheat moisture, per cent	Absorption, per cent	Gluten			Wt. of loaf, gm.	Loaves per barrel	Vol. of loaf, cc.	Texture of bread in order of excellence
				Per cent	Color	Quality				
JENKINS CLUB										
No. 1, 15 days after planting	11.50	....	53	28.7	Yellow	Soft	500	284	1950	2
No. 2, 45 days after planting	13.45	12.2	53	33.6	Yellow	Soft	495	284	1900	4
No. 3, 75 days after planting	13.80	12.4	53	34.5	Yellow	Soft	496	284	1750	5
No. 4, untreated but adjacent to No. 3	10.35	12.5	53	25.8	Yellow	Soft	500	284	1800	1
No. 5, untreated	9.80	12.6	53	24.5	Yellow	Very soft	493	284	1800	3
PUSA										
No. 1, 15 days after planting	13.50	12.7	61	33.7	Yellowish	Good	500	298	2120	1
No. 2, 45 days after planting	15.05	12.7	62	37.6	Yellow	Very good	502	300	1960	3
No. 3, 75 days after planting	15.05	12.6	63	37.6	Yellowish	Good	505	300	1970	2
No. 4, untreated but adjacent to No. 3	12.40	13.0	65	31.0	Yellowish	Soft	507	303	1970	4
No. 5, untreated	12.60	12.6	65	31.5	Yellowish	Soft	506	303	1950	5
SONORA										
No. 1, 15 days after planting	11.25	12.3	58	28.1	Yellow	Soft	500	293	1950	2
No. 2, 45 days after planting	10.90	12.6	58	27.2	Yellow	Soft	500	293	1900	3
No. 3, 75 days after planting	11.35	12.1	57	28.4	Yellow	Good	498	291	2120	1
Nos. 4 and 5, untreated but adjacent to No. 3	8.65	....	57	21.6	Yellow	Soft	505	291	1800	4

This array of wheat may be divided into two groups: (1) samples of the same variety that differed as to the percentage of protein in the grain, but which ripened together; (2) samples of the same variety that did not ripen together, but were alike as to the percentage of protein in the grain. The differences obtained, however, among the samples, were not so large as they would have been under ideal conditions. The differences in the length of the period of ripening were not so pronounced as they probably would have been had planting been earlier, and the heading out of the grain would have occurred at a more favorable season than mid-summer. However, it is thought that the results obtained are worthy of presentation and that they show an important relationship between the quality of the flour and two factors: (a) the percentage of protein in the grain, (b) the length of a certain period of development that expressed itself in differences in ripening.

The data given in Table II were worked out in the laboratory of the Sperry Flour Company, Vallejo, Calif. The writer is indebted to C. B. Kress, chief chemist, for his co-operation and the use of his laboratory; also to Miss Fanny Johnson and Robert Knudson, respectively, for the baking and chemical operations.

The chief features of the two tables are:

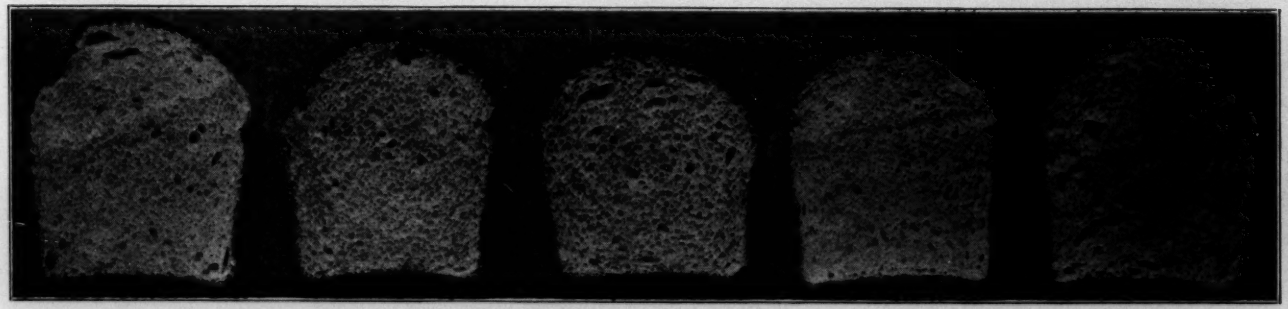
1. In every case where nitrogen was supplied to wheat in the field, 45 or 75 days after planting, the grain was decidedly higher in protein than was that obtained from wheat which was either not fertilized with nitrogen or was fertilized at the time of planting. The responses of Pusa and Sonora differed from that of the others in that the percentage of protein in the grain did not increase with later applications of nitrogen. As this pertains to the physiological characteristics of varieties, it will not be discussed here.

2. In Bunyip and Hard Federation, loaf volume increased proportionally with increase in percentage of protein of the grain. In Jenkins Club, loaf volume decreased with increased percentage of protein in the grain. In Early Baart and Pusa, the greatest loaf volume came from the wheat that received nitrogen at the time of planting. In Sonora, the greatest loaf volume came from wheat that received nitrogen when the plants were about two months old.

3. The peculiarities of the loaf volume and the percentage of protein of the different wheats correspond in a rather general way with the peculiarities of the varieties in their response to applications of nitrogen at various growth stages of the plant.

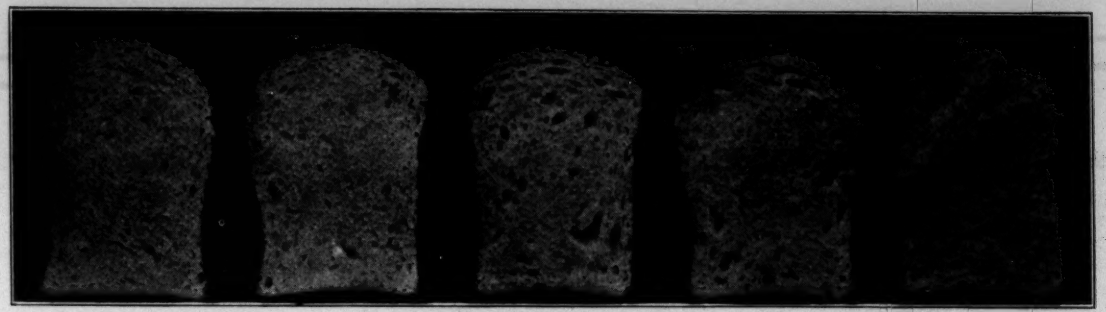
Early Baart

No.	1	2	3	4	5
Protein.....	11.8	13.1	13.8	12.3	12.1
Wet crude gluten.	29.5	32.7	34.5	30.7	30.2
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none	none



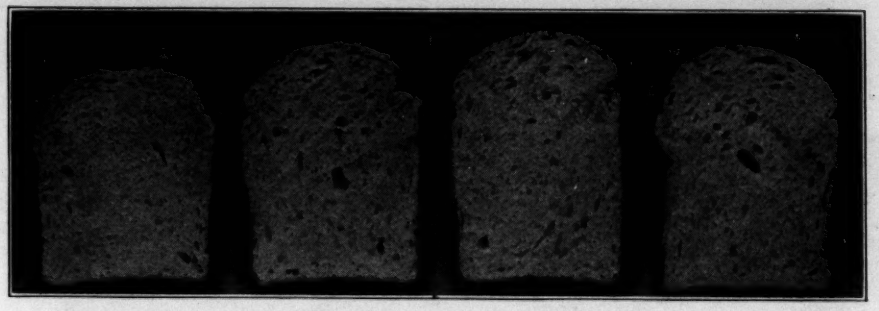
Jenkins Club

No.	1	2	3	4	5
Protein.....	11.5	13.45	13.8	10.35	9.85
Wet crude gluten.	28.7	33.60	34.5	25.80	24.60
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none	none



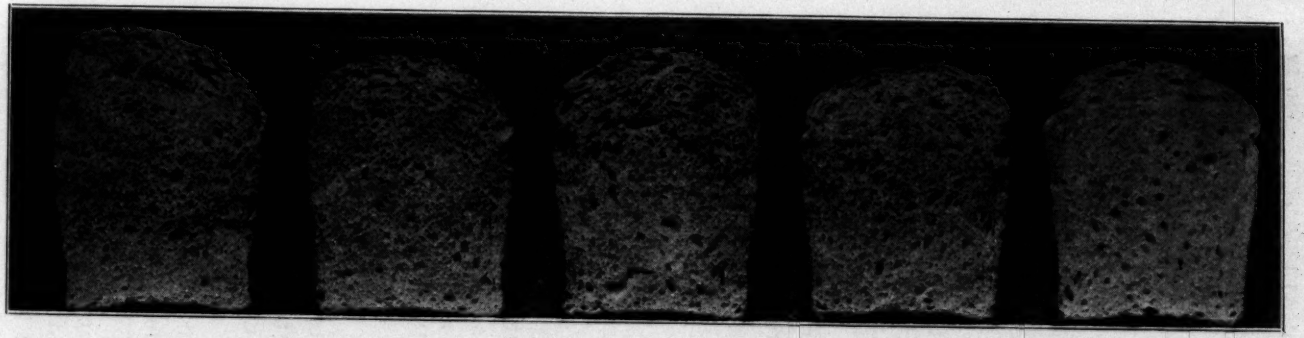
Bunyip

No.	1	2	3	4	5
Protein.....	12.3	13.9	15.1	12.4	12.35
Wet crude gluten.	30.7	34.7	37.7	31.0	30.8
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none	none



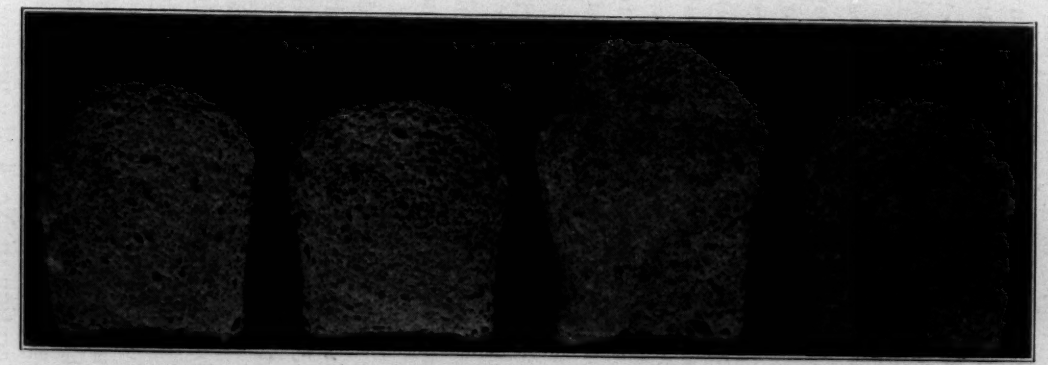
Hard Federation

No.	1	2	3	4 & 5
Protein.....	11.75	13.00	14.15	10.60
Wet crude gluten.	29.3	32.5	35.3	26.5
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none



Pusa

No.	1	2	3	4	5
Protein.....	13.5	15.05	15.05	12.40	12.60
Wet crude gluten.	33.7	37.6	37.6	31.0	31.5
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none	none



Sonora

No.	1	2	3	4 & 5
Protein.....	11.25	10.90	11.35	8.65
Wet crude gluten.	28.1	27.2	28.3	21.6
Nitrogen applied.	15 days after planting	45 days after planting	75 days after planting	none

That differences in the length of a certain period of development and expressed in these experiments by differences in ripening of the samples of any variety, markedly affected the quality of the flour, is now presented as the main thesis of this paper. Whenever two samples of wheat from the same variety and having the same percentage of protein in the grain, did not produce bread equal and alike in quality, the poorer invariably came from the wheat which ripened first.

Considering each variety separately in the light of the relations above indicated, the order of merit of the bread of Bunyip is 3–2–1–4–5. This is also the order of the excellence of the wheat in respect to the percentage of protein of the grain. Samples 4 and 5, the untreated ones, are clearly inferior to Nos. 3 and 2. They are not, however, markedly inferior to No. 1. The slight difference in merit between the two untreated samples is in favor of No. 4, which was adjacent to a treated plot and reflected slightly the result of the nitrogen supplied to No. 3. The slight progressive improvement in quality of the bread produced from the wheats supplied with nitrogen at correspondingly later growth periods and having a progressively higher percentage protein in the grain, is consistent, but not pronounced, and is accounted for by the fact that the difference in the maturity of several samples, as shown in Table I, is not large. The relationship, however, becomes clearer in the subsequent discussions.

The order of merit of Early Baart as to loaf volume is 1–4–5–3–2 and as to texture, 1–4–5–2–3. Both the best and the poorest bread came from wheats fertilized with nitrogen, respectively, Nos. 1, 2, and 3. The excellence of No. 1, which was appreciably lower in protein than the untreated ones and markedly lower than the other treated ones, presents a peculiar problem. It is to be noted that the five samples ripened on the same day, differing in this feature from Bunyip.

The order of merit in Hard Federation as to loaf volume, is 3–2–4–1 and as to texture, 2–3–1–4. Omitting, for the time being, consideration of No. 4, the untreated one, it is to be noted that the quality of the bread is related in a general way to the factor usually assumed to indicate the quality of a flour, namely, the protein content of the grain. Sample No. 4 is superior to No. 1 in loaf volume, but markedly lower in percentage of protein in the grain. It is to be noted that No. 4 ripened eleven days later than No. 1, and hence it is assumed that its inferiority was produced by that factor. The slight superiority of No. 2 over No. 4 is accounted

for in the markedly higher protein content of the former sample, but the failure of No. 2 to be markedly superior to No. 4 is due to the fact that the beneficial effect ordinarily associated with such high protein value in grain was nullified by the difference in length of the period of ripening, which was eleven days in favor of No. 4.

The data on Jenkins Club are: loaf volume, 1–2–4–5–3 and texture, 4–1–5–2–3. All samples ripened together, and the failure of the high protein values of the treated wheat to produce corresponding results in volume of loaf, lies in the factor associated with and expressed by the period of ripening. The smaller loaf volume produced by No. 4 and No. 5, as compared to No. 1, is due to the lower protein content of untreated samples.

Jenkins Club was decidedly the poorest of the six varieties. It is a very late wheat. Altho it ripened later than any of the others, it is contended by the writer that it ripened much too soon to produce grain that would yield a high-quality flour; and that the poorness of this extremely late wheat was augmented by the failure of processes associated with a period of development expressed in the length of the ripening period, to function sufficiently. In the case of Jenkins Club, as with all other varieties, the grain was well filled; comment having been made on its apparent physical excellence by several experts.

Pusa is an early wheat. Sample No. 1 is decidedly the best of the lot, in both loaf volume and texture. The rest of the set are practically equal as to loaf volume. The poorest, as to texture of the bread, was that of the untreated wheat. However, if No. 1 is excluded, the chief feature that distinguished Pusa from the other varieties was the lack of any decided differences among the samples as to both loaf volume and texture. This is explained by the fact that variation in the period of ripening cannot be so large in an early wheat as in a late wheat. Samples Nos. 2 and 3 were decidedly higher in protein than Nos. 4 and 5, and the failure of these characteristics to express themselves in the bread must be ascribed to the factor indicated in Table I on the maturity of the plants. All samples ripened at the same time, hence the brevity of the period of ripening of the samples that received nitrogen one or two months after planting not only nullified but impaired the beneficial effect that conceivably should have accrued from such markedly high-protein grain. Two questions may be asked at this point—Why was not bread from No. 3 of poorer quality than that from No. 2? Why was the effect of the equality in ripening among the samples of Pusa not so pronounced as in

Jenkins Club? The answers lie in earliness of the variety. The growth stage in Pusa, defined by one month after planting, caused the greatest depression in quality of the bread, or, it may also be termed the high point of sensitivity to the factor causing the characteristic relations herein discussed. The analogous relation in the late variety Jenkins Club was obtained two months after planting. (It is to be understood that these time intervals are not given as being absolute and fixed values of characteristics of these varieties.)

The best loaf of bread, as to both texture and loaf volume, among the samples of Sonora, is No. 3. The poorest is No. 4, the untreated one. The order of excellence in this variety is 3–1–2–4, as to both loaf volume and texture. The results obtained from Nos. 3 and 4 are expected and in accordance with the character of the grain from which they were produced. Sample No. 1 has the same percentage of protein in the grain as has No. 3 and is decidedly inferior to No. 3. However, the data on the maturity of the samples clarify these results. Sample No. 3 ripened 10 days after No. 1. The difference in quality of the bread between Nos. 1 and 2 is accounted for by the difference in percentage of protein of their grain, there being no difference in their relative maturity. Sonora is notoriously poor as to the desirable property of strength in flour. Occasionally and for some unknown reason, Sonora is known to produce a very strong flour. Sample No. 3 was pronounced by the experienced millers and bakers as being equal in quality to any bread produced from the best northern hard spring wheat, and this wheat was not high in protein, altho the value obtained is fairly high for the variety. The excellence of sample No. 3, not only among the samples of Sonora but among those of all varieties, is a striking example of the fact that quality of flour is more than a simple relation of the protein content of the grain. It appears that in addition to the protein factor, the quality of flour is related to processes that occur during a period of development of the plants that is expressed by differences in the length of the period of ripening.

From the above the conclusion is derived that the "strength" of flour of any variety of wheat (and obviously "strength" of flour varies with variety) is factorially related to (1) the protein content of the grain and (2) some factor or process connected with a period of development of the plants which is reflected in differences in time of ripening. In this investigation, this difference varied from two to fourteen days. Whether or not the improvement in

the later maturing samples occurred during the actual period of ripening [as this stage of development is defined by Brenchley and Hall (1909) to be the period characterized by the desiccation of the grain] cannot be stated. In view of the work of these authors and that of Woodman and Engledow (1924) it does not appear that the beneficial effect obtained, if at all connected with the period of ripening, was confined exclusively to that period. The period when this improvement occurred, however, probably was not longer than the number of days that the maturity of any sample was delayed, as the sample that showed the greatest benefit from delayed maturity was one that received nitrogen ten weeks after planting—the most advanced stage of growth at which the fertilizer was supplied.

As variation in the quality of the bread produced from the various samples of any of these varieties is accounted for in protein content of the grain and the differences of ripening, the question may now be asked: How are these factors related?—or, what property, if any, has been imparted to the protein of the grain during the longer period of growth, that was not imparted during the shorter one? Granted that the beneficial effect of delayed maturity operated through the nitrogenous constituents of the grain, then the difference in the quality of bread as obtained conceivably should be correlated with differences in the distribution of various nitrogenous compounds in the grain. As studies have been organized by the writer to follow the development of the grains of wheat of several varieties that are to receive nitrogen at various stages of their development, no speculation will be attempted as to what caused the results obtained. The writer cannot forego a brief comment on the finding of the English investigators mentioned. That wheat plants cease to absorb nitrogen from a week to two months before the ripening of the grain has been shown by several investigators. The cessation of assimilation of nitrogen is dependent either upon its absence in available form in the soil, or by the state of development of the plants. The application of available nitrogen to the plants at an advanced stage of development provides for the absorption of relatively large amounts of the material during the period of the development of the grain,[2] and as shown by Woodman and Engledow (1924) a very large percentage of nitrogen in the grain during the early part of the period of formation is non-protein. As the absorption of nitrogen can be markedly increased by late application of nitro-

[2] In certain experiments by the writer, more than four-fifths of the total amount of nitrogen contained in the grain of wheat was absorbed by the plants during the last three weeks of growth.

gen to the plants, and in view of findings of Brenchley and Hall and Woodman and Engledow, that considerable time is required for the transformation of non-protein nitrogen into protein nitrogen, it seems not improbable that the samples which produced bread of poor quality could have contained relatively high amounts of non-protein nitrogen. Whether or not this was detrimental to the quality of bread cannot be stated. It follows that if the percentage of non-protein nitrogen is high, the reciprocal percentage of protein nitrogen is consequently relatively low. It may have been that the amount of gliadin and glutenin was much lower in the grain that was high in total nitrogen, produced by the late application of nitrogen to the plants, than in grain low in total nitrogen. A word may also be said respecting the findings of Woodman (1924). He found that strong wheat may synthesize one type of glutenin and weak wheat a different type. Since this protein is considered to be the most important nitrogenous constituent in wheat grain in respect to quality of flour, it is conceivable that differences in the ripening of the grain may have been reflected in the properties of the glutenin. One form of protein may result from a short period of development, another form from a long period; but whatever the cause in the property of the proteins involved, it does not appear improbable that the poorness of some of the grain high in total nitrogen was in part due to the over-feeding of the plants; and that the ill effects of this over-feeding can be overcome by prolonged growth by which it is transformed into the proper protein.

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WHEAT AND FLOUR STUDIES X. FACTORS INFLUENCING THE VISCOSITY OF FLOUR-WATER SUSPENSIONS I. EFFECTS OF TEMPERATURE, DEGREE OF HYDRATION, AND METHOD OF MANIPULATION[1]

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(Read at the Convention June 11, 1926)

Introduction

In the work which has been done on the viscosity of wheat flours, difficulty has been experienced in obtaining identical results for the same flour in regard to absolute viscosity when the viscosity determinations were made over a period of several days. Gortner (unpublished data) noted, however, that altho the absolute viscosities as determined at different times did not agree, nevertheless the tangents of the angles obtained when the logarithms of the viscosities were plotted as ordinates and the logarithms of the flour concentrations as abscissae, were the same when series of viscosity determinations obtained at corresponding times were used in plotting the lines. For example, on one day, concentrations of 15, 18, and 21 grams of flour might show viscosities of 40, 60, and 90 degrees MacMichael when the viscosities were determined by the method outlined by Gortner (1924); while at another time, under apparently identical conditions, these concentrations of the same flour would show viscosities of 44, 66, and 99 degrees MacMichael. When the tangent of the angle is all that is required, it makes no difference what the individual viscosities are. When, however, it is desired to check with different laboratories or to make viscosity determinations over a period of time to determine, for example, the rate of or changes in proteolytic activity in flour according to the method used by Collatz (1922), then changes in the absolute viscosities become the criteria for judging proteolysis.

A method for determining proteolytic activity based on viscosity methods appears to have several advantages over other methods which might be used, especially when the rate of proteolysis is likely to be slow. Swanson and Tague (1916) used the formol titration method with apparently satisfactory results, but

1 Published with the approval of the Director.

as the rate of proteolytic activity was slow in the flour extracts which they used, several weeks were required to determine the rate of proteolysis. Sharp and Elmer (1924) attempted to measure proteolytic activity in flour milled from frozen and non-frozen wheat by determining the protein in fractions soluble in 5% potassium sulphate and in 70% alcohol, and by determining the fraction present in the residue after these two extractions when flour suspensions were auto-digested for several weeks. When wheats were harvested at different stages of development and portions of the samples were subjected to light frost artificially, no significant difference appeared in the protein fractions whether determined on flour milled from frosted or non-frosted wheat. Neither do any significant differences appear in the amino nitrogen content of the frozen and non-frozen wheat flours when the amino nitrogen is determined in flour suspensions after varying periods of auto digestion. The viscosity method seems to offer a means of measuring more subtle changes than those which depend on the splitting of the protein molecule to the amino acid stage. As evidence of this the following experiment taken from research along another line will be cited. A suspension of 20 grams of flour in a liter of water was prepared (1 cc. of toluene was added as a preservative). To this suspension was added 1 cc. of juice expressed from green wheat leaves. At the end of 11 hours the liter of water was decanted and the suspension washed with an additional 500 cc. of water. After allowing it to stand for 15 minutes, the 500 cc. of water was decanted and the viscosity of the suspension (acidulated with 0.50 cc. of 20% lactic acid) was determined, using a MacMichael viscosimeter. The viscosity at this time was 34 degrees MacMichael. After 24 hours of auto digestion and similar treatment, the viscosity decreased to 4 degrees MacMichael, which is practically the viscosity of the non-acidulated suspension. The viscosities of flour suspensions digested for 1 hour, 11 hours, and 24 hours, under identical conditions but not containing wheat leaf juice, were 178, 158, and 133 degrees MacMichael, respectively. From these values the effect of the proteases of the wheat leaf juice may be seen. The amino nitrogen in 2 cc. of the liter of water decanted from the suspension containing leaf juice was 0.008 cc. greater after 11 hours of auto-digestion than in an equal quantity of the freshly prepared decantate, while at the end of 83 hours it was only 0.025 cc. greater. In the suspensions containing no leaf juice, no increase in amino nitrogen was found even tho the auto-digestion was allowed to

proceed for 83 hours. These small differences in quantity of amino nitrogen and the relatively large error in their measurement indicate that some method other than the Van Slyke might reveal more definitely the changes in the protein molecule which probably occur before the end products of protein digestion begin to appear. A method based on the use of viscometry appears to offer advantages in this regard.

Since it is proposed to study differences in proteolytic activity between flours milled from frosted and non-frosted wheat by the viscosity method, an investigation was necessary to determine the causes for the different viscosities obtained at different times. The necessity for this study became evident in some of the preliminary work. A suspension was prepared using 20 grams of flour and a liter of water, and was allowed to stand for 24 hours at room temperature. A cubic centimeter of toluene was added to prevent bacterial action. At convenient intervals the flask was shaken. At the end of 24 hours the flask was shaken, allowed to stand for 15 minutes, the water decanted, and an additional 500 cc. of water added. Fifteen minutes later this water was decanted, the residue made to 100 cc. and the viscosity of the acidulated suspension determined. The viscosity in this instance was 225 degrees MacMichael. When an identical procedure was followed except that the suspension was allowed to stand for 1 hour with shaking at 10-minute intervals, a viscosity of 174 was obtained. Thus it is evident that certain phenomena were operating in the flour-water suspension to mask any proteolysis which might have occurred. It is chiefly with a study of these phenomena that this work is concerned.

Experimental

Viscosity of Extracted Flour-Water Suspensions

Ostwald (1913) states that the viscosity of substances in the colloidal state is affected by (1) concentration, (2) temperature, (3) degree of dispersity, (4) solvate formation, (5) electric charge, (6) previous thermal treatment, (7) previous mechanical treatment, (8) inoculation with small quantities of more viscous liquids, (9) time or age, and (10) additions of either electrolytes or non-electrolytes. When flour suspensions are used for studying the viscosity of colloids, most of these factors play a part, but all of them are subject to quite definite control. In the present study the effects of some of them on the viscosity of flour suspensions will be considered.

In addition to the factors mentioned by Ostwald, several others must be considered in the determination of the viscosity of flour-water suspensions prepared according to the method of Gortner (1924), in which the electrolytes are extracted by preparing a suspension of the flour in one liter of distilled water. The suspension is shaken at 10-minute intervals for a period of 45 minutes, let stand for 15 minutes and the supernatent liquid decanted. The residue is again extracted with an additional 500 cc. of water. In addition to the effect of the temperature of the wash water on the colloids themselves, it is likely that the temperature affects the rate at which the enzyme phytase acts on the phytin. The more rapid is this activity the more electrolytes are removed in the washing. Collatz and Bailey (1921) have shown that for temperatures up to 60° the conductivity of water extracts prepared from wheat flours increases. This indicates a lower ash content in the residue. It is also conceivable that the degree of mechanical treatment would affect the rate of activity of this enzyme and thus alter the electrolyte content of the final suspension, the viscosity of which is to be determined.

Degree of dispersity and solvate formation are factors which the writer believes have received too little attention in the viscometric studies in which flour has been used. When the same flour is used, it is obvious that degree of dispersity is not a factor. However, when samples of the same wheat are milled on different mills, flours of different fineness may result, with consequent differences in the degrees of dispersity of their water suspensions. Studies of such nature offer interesting possibilities. As regards solvate formation, Johnson and Bailey (1924) have shown by means of the Chopin extensimeter that flour with sufficient water to wet it becomes more completely hydrated when allowed to stand from 4 to 6 or even 24 hours. It is possible that a longer period of time in contact with the water or a higher temperature of the water will hasten solvation and thus affect the absolute viscosity of a flour-water suspension.

Since some of the protein responsible for viscosity may be removed by imperfect decantation, this suggests a reason for the differences in viscosity obtained on the same flour. A simple determination of the protein in the extract would indicate how serious a cause of error this might be. Evidence will be presented later to show that error due to this cause is not very serious.

At the beginning of the work it was thought that the chief reason for the discrepancies in absolute viscosity manifest when

the viscosity was determined on successive days, was due to difference in mechanical manipulation. Further observation, however, indicated that the temperature at which the extraction was conducted was probably a more important factor. Consequently 14-, 16-, 18-, and 20-gram samples of flour were extracted with distilled water at various temperatures from 15° to 96° C. according to the method of Gortner (1 hour plus 15 minutes) and the viscosity of the residue in a volume of 100 cc. was determined following acidulation. The extraction flasks were kept in a water bath the temperature of which was carefully controlled. Viscosities were determined at room temperature. It was necessary to cool or warm the suspensions, as the case might be, to allow them to attain this temperature, as it was found that the temperature at which the viscosity determination was made had considerable effect on the viscosity. A cold suspension resulted in a high viscosity while a warm one gave too low a viscosity. Care was also taken to shake each flask in a uniform manner in order to control as far as possible the mechanical treatment.

The results obtained in this study are given in Table I. From these data it is evident that the temperature of the water used in extracting the electrolytes has a marked effect on the viscosity of the flour-water suspension obtained. In accordance with Gorner's observation, the constants b calculated from the viscosities at any given temperature are practically the same at temperatures below 50° C. Thus it is quite likely that the difference in viscosity shown by the same flour at different times may be due to differences in temperature of the water used in extracting the electrolytes. The difference in viscosity between the 20-gram sample extracted at 20° C. and that extracted at 30° C. is 71 degrees MacMichael. This means that for each degree of difference in temperature there is a difference in viscosity of 7° MacMichael. It is quite possible that the temperature of the distilled water kept in the laboratory may vary sufficiently to account for the discrepancies in the viscosities of flour suspensions which have been noted.

In Table II are given the factors for converting the viscosity at one temperature to the viscosity at another temperature through a series of temperature ranges. These factors for the different flour-water concentrations check very well, as will be noted in the data. It is also to be noted that the factors for converting the viscosities determined at temperatures below 30° to those at higher temperatures up to 30° are greater than those for con-

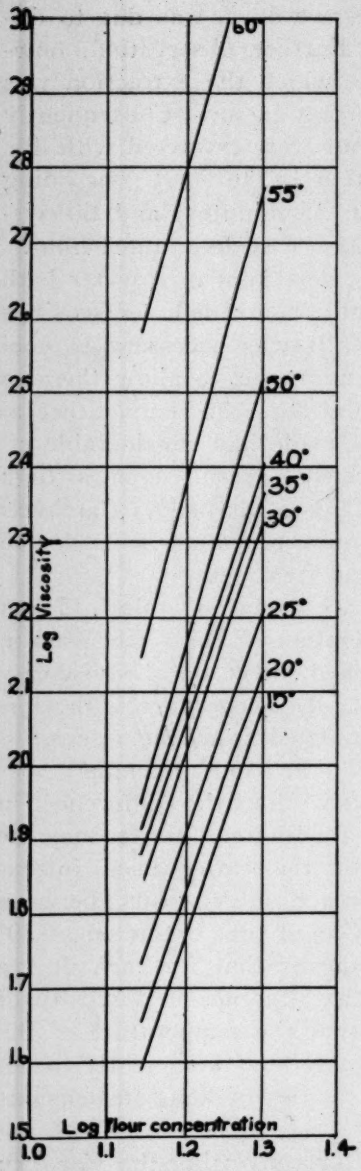

Fig. 1. Logarithmic Viscosity-Concentration Curves of the Flour Plotted from Viscosity Determinations Made at Different Temperatures.

verting through an equal temperature range above 30°. This, however, does not hold for temperatures above 50° C. Evidence for this fact is presented graphically in Figure 1, in which the logarithms of the flour concentrations are plotted along the axis as abscissae and the logarithms of the viscosities along the axis as ordinates. A series of lines is obtained, each line representing viscosities determined at a particular temperature. The distance apart of these lines represents the factor for converting the viscosity at one temperature to that at another temperature. Thus the distance apart of lines plotted from data obtained at 15°, 20°, 25°, and 30° C. is greater than for lines plotted from data obtained at 35° and 40° C.

In Figure 1 it may be seen also that a family of parallel lines is obtained as long as the temperature of extraction is below 50°. This fact is also apparent from a consideration of the constant *b* data. At 50° C. there is an increase in this value and it continues to increase as the temperature of extraction is raised. This phenomenon will be considered later in the discussion.

While the constant *b* remains practically the same when the temperature of extraction is below 50° C., constant *a* increases (becomes less negative) throughout this temperature range. Since *b* remains the same and we know the factor for conversion

from the viscosity at one temperature to the viscosity at another temperature, if we know the value for a at one temperature, it should be possible to calculate a at any temperature. The equation for the series of lines graphed in Figure 1 is:

log. viscosity=log. a + b (log. concentration)

where a determines the position of the line on the viscosity axis and b is the tangent of the angle which the line makes with the horizontal.

If we determine the temperature at which a given flour is extracted, the log. viscosity also becomes a constant. Then a may be obtained for any temperature by the equation

log. viscosity=log. a + (log. conversion factor) + b (log. flour concentration)

In order to make this better understood an example will be given. Constants b at temperatures 20° and 40° happen to be the same, therefore the constant a at 40° will be calculated from the data obtained at 20°. The factor for converting the viscosity at 20° to the viscosity at 40° is 1.745.

Applying the equation

log. viscosity=log. a + (log. 1.745) + b (log. flour concentration)

log. constant a at 20°= −1.95

log. 1.745= 0.2418

calculated a= −1.7082

Thus the calculated value for a agrees very well with the value 1.70 obtained experimentally.

The data from which these calculations were made are included in Tables I and II.

In order to determine the effect of temperature on the quantity of electrolytes removed by extraction with water and to correlate this factor, if possible, with the higher viscosity at higher temperatures of extraction, the resistivities of the 1000-cc. decantates were determined. These data are given in Table III, and they show very definitely that at the higher temperatures of extraction greater quantities of electrolytes are removed. This indicates, obviously, that at lower temperatures greater quantities of electrolytes remain in the residues which are used for making the viscosity determinations. It is probable that electrolytes themselves may not be present, but substances capable of allowing them to form.

Since it was possible that the substances capable of giving rise to electrolytes might be present in the decantate but not yet

converted to electrolytes, portions of the decantates, preserved with toluene, were stored for 168 hours. At the end of this time the resistivities of the extracts were again determined. These data are also given in Table III. While an elaboration of more electrolytes does occur in the stored decantates, yet the order of the resistivities after 168 hours is practically the same as the order of the resistivities of the fresh decantates. Hence it is evident that flour suspensions extracted at lower temperatures contain more material capable of elaborating electrolytes than suspensions extracted at higher temperatures. It is probably these materials which result in the lower viscosities obtained when water at the lower temperatures is used in preparing flour suspensions for viscosity determinations.

If the higher viscosity of suspensions extracted at higher temperatures were due to more rapid phytase activity at the higher temperature, with consequent removal of more electrolytes, then a longer period of digestion of the flour-water suspension before decantation of the wash water should accomplish the same purpose, i. e., should remove more electrolytes. The longer period of digestion would obviously give the phytase a longer period in which to produce electrolytes that would be decanted. Eighteen-gm. portions of flour were accordingly extracted with the same water at 20° and at 40° C. for varying periods of time. The viscosities of the resulting suspensions were determined in the usual way and the resistivities of the 1000-cc. decantates as well as those of the 500-cc. decantates were determined. These data are given in Table IV. A study of these data indicates clearly that the longer periods of digestion (up to 5 hours for the suspensions digested at 20° C.) produced suspensions the resultant viscosities of which were higher than those digested for a shorter time. In the suspensions digested at 40°, there was an increase in viscosity for the 2-hour digested suspension, but the viscosity decreased quite regularly when the time of digestion was increased. The same phenomenon occurred after 5 hours for the suspensions digested at 20° C. The reason for this decrease in viscosity is not known. It may be due to effects of bacterial or proteoclastic activity beginning to become manifest, or to solvation of some of the proteins responsible for viscosity and their subsequent decantation due to solvation.

From the data on resistivity it is to be noted that as the time of extraction increases resistivity decreases and continues to decrease as long as viscosity increases. When the viscosity ceases

to increase, however, resistivity becomes practically a constant. This is the case for suspensions extracted at either 20° or 40° C.

As regards the resistivities of the 500-cc. decantates, little significant difference appears in those obtained at 40°. For the resistivities of the 500-cc. decantates obtained from the suspensions extracted at 20°, however, there is a definite increase as the time of extraction is increased. This indicates that more and more of the substances responsible for electrolyte production are hydrolysed and decanted with the first liter of decantate. The inverse relation between period of extraction and resistivity of the 1-liter decantates obtained at 20° substantiates this idea.

A point worthy of attention in considering the data in Table IV is that there is considerable difference in the maximum viscosity obtainable at 20° and at 40°. The maximum viscosity obtained at the end of 5 hours at 20° C. was 162 degrees, while that obtained at the end of 2 hours at 40° C. was 205 degrees. The difference in resistivity after these periods of extraction was less than 9 ohms. The resistivity of the 500-cc. decantates was the same. The writer does not consider the difference in electrolytes responsible for this difference in resistivity sufficient to account for the difference in viscosity which existed — 43 degrees MacMichael. It is probable that the temperature to which the flour proteins have been exposed is responsible for a part of this large difference in viscosity. Sufficiently definite data, however, are not yet available to settle this question.

The data in Table V also may have certain significance in regard to solvation or hydration. For example, the resistivities of the decantate maintained at 15° C. for 4 hours and that maintained at 25° for 1 hour are nearly the same (791 and 794 ohms, respectively) yet the viscosity of the first was 209 as compared with 165 for the second. From this table other comparisons may be found relating to the effect on viscosity of time of extraction, resistivity of suspension, and temperature of extraction. As has been stated, no organized effort has been made in this study to separate and evaluate the various factors which influence hydration.

In Table V are presented additional data which show the effect of time of extraction on the resistivity of the decantate and on viscosity. The conclusions are the same as those drawn from the data in Table IV.

Sharp and Gortner (1923) extracted flours with increasing quantities of distilled water and in one case obtained the highest viscosity when 12 portions of water of 500-cc. each were used. In

view of the effect on the viscosity of the temperature of the water used in extracting electrolytes, it was considered worth while to extract with successive quantities of water at several temperatures. Eighteen-gram portions of flour were accordingly extracted with from 1 to 11 liters of water at 20° and at 40° C. The results are given in Table VI. In the case of the flour extracted with water at 20°, the viscosity increased quite regularly from 101 degrees MacMichael for flour washed with 1 liter of water, to 180 degrees for flour washed with 9 liters of water. The viscosities of the flours extracted at 20° C. with 10 and 11 liters of water were slightly lower than that of the flour extracted with 9 liters. In the flour extracted with water at 40°, the viscosity became practically constant after washing with 3 liters of water, and remained so until washed with 7 and 8 liters. It is quite likely that the decrease in viscosity for both the flour extracted at 20° and that extracted at 40° is due to imperfect decantation, as with from 8 to 11 separate decantations the possibility that some of the protein responsible for viscosity may be decanted is considerable. It was hoped that by long-time digestion or continued washing the maximum viscosities attained by the same flour concentration at any temperature might be the same. In the work recorded thus far, this has not been capable of demonstration. From the data in Table VI the maximum viscosity of the flour suspension extracted at 20° C. is observed to be 180 degrees, while for that extracted at 40° C. it was 224 degrees. It has already been noted that for flour digested for from 1 to 8 hours the maximum viscosity for the suspension digested at 20° was 162 degrees, while for that digested at 40° C. it was 205. Thus the continued extraction produces suspensions of higher maximum viscosity than does continued digestion, whether the treatment be at 20° or at 40° C. Hence the viscosities at these two temperatures are no nearer being the same, regardless of these two treatments.

Data have been presented which indicate the importance of controlling the temperature at which flour suspensions are extracted. Other factors, also, are worthy of consideration, among them being the degree of accuracy with which the decantation can be made. The viscosity determinations recorded in Table I were made in duplicate. Consequently, 100-cc. portions of the 1000-cc. duplicate decantates were taken for nitrogen determinations. The duplicates all checked within limits quite acceptable for this work. Hence the degree of decantation is sufficiently

accurate to make possible viscosity results which would check from day to day.

Data showing the quantity of nitrogen extracted in the first liter of extract are given in Table VII. A study of this table indicates that as the temperature of extraction is raised the quantity of nitrogen extracted increases. When the temperature of extraction was 80° C., however, there was a decrease in the quantity of nitrogen in the extract. When the temperature of extraction was raised to the boiling point, there was a still further decrease in the quantity of nitrogen removed. Thus, when 16 grams of flour was extracted with water at 70°, 14.49 centigrams of nitrogen was extracted; at 80°, 12.54 cg.; at 96° for 1 hour, 10.66 cg.; and at 96° for 3 hours, 7.39 cg. The higher temperatures probably result in the coagulation of some of the proteins, and the time of exposure to the higher temperature is apparently one of the factors involved in the coagulation.

Sharp and Gortner (1923) found that when practically all of the gliadin had been removed from a flour suspension the highest viscosity was exhibited. For this reason they attributed the viscosity of the suspension to the glutenin fraction of flour proteins. They further suggest that gliadin may have a depressing effect on viscosity. If this be true, viscosity is a function of both the quantity of gliadin removed and the electrolytes removed; and from the data available it is evident that both factors are related to the temperature of extraction. Thus, from a liter of suspension containing 14 grams of flour, 24.8% of the nitrogen was extracted at 15° C. while 30.9% was extracted at 35° C. Moreover, as the ratio of flour to water in suspension was increased, the percentage of nitrogen extracted decreased. Thus, when 20 grams of flour per liter of suspension was extracted at 15° and at 35°, 22% and 26.1% of the nitrogen was removed, respectively, while under identical conditions but using 14 grams of flour for extraction, 24.8 and 30.9% of the nitrogen, respectively, was removed. The effect of gliadin on the viscosity of glutenin, however, is a subject which demands further research before definite conclusions can be drawn. The writer believes that the removal of electrolytes is chiefly responsible for the higher viscosity when higher temperatures of extraction are used.

Another factor which appeared to affect the viscosity of flour-water suspensions was the mechanical manipulation of the suspension during the extraction of the electrolytes. For this reason

two variations were made in the usual procedure of extracting the electrolytes. In one case bottles of approximately one-liter capacity were filled with the suspension and placed in a rotary shaking machine for 45 minutes and thereafter treated in the usual way. In this way it was possible to keep practically all the flour in suspension during the whole extraction period without too drastic physical treatment of the material in suspension. In the other case bottles of two-liter capacity were used and the one liter of suspension placed in them. Then a few drops of capryllic alcohol were added to prevent foaming and the flasks were rotated continually for 45 minutes. As the flasks were not full, the suspension was churned about vigorously. After shaking, the suspension was treated in the regular way.

The effects of different methods of treatment were studied at temperatures of 20° and 35° C. It was obviously not possible to control the temperature so accurately when the shaking machine was used. The room in which the work was done was maintained at these temperatures as closely as possible and the temperatures of suspensions may have varied by as much as 2 degrees during the extraction period. It was believed, however, that the results would indicate definitely enough the effects of temperature on the treatment.

It was hoped that information could be obtained in this manner concerning the effects of manipulation on the viscosity of the suspension. The protein content of the decantate and its resistivity were determined. Results are given in Tables VIII, IX, and X. The data in Table VIII indicate again that the effect of the higher temperature of extraction was to increase the viscosity. It was also clear that the degree of mechanical treatment affected the viscosity. The viscosity of the 20-gram suspension contained in the continually shaken bottle, half full, was 294 degrees, the viscosity of the correspondingly shaken suspension contained in the completely filled bottle was 236 degrees, while that of the flask shaken at 10-minute intervals was 230 degrees. The same order of viscosity obtained regardless of temperature of extraction or of flour concentration.

A study of the constant *b* data indicated that the most vigorously treated flour had the highest value. It may be that the degree of treatment affected the flour proteins in a harmful manner and that a relatively larger proportion was thus affected at the lower flour-water concentrations. This would account for a higher value for *b*. In order to test this further, 14-, 16-, 18-, and 20-

gram portions of the flour suspended in 1000 cc. of water were shaken continually for 7 hours in half-filled bottles. At the end of the shaking period and after allowing the suspensions to stand for 15 minutes, the supernatant liquid was decanted and the flour extracted with an additional 500 cc. of water for 15 minutes. After decantation the suspensions were made to 100 cc. and the viscosities after acidulation were determined. Viscosity determinations were also carried out on a series of suspensions prepared in a similar manner except that they were shaken at 10-minute intervals during the 7-hour period instead of continually. The data obtained from these experiments are given in Tables VIII and IX.

The data in Table VIII indicate, again, that in most cases the more vigorously the flour suspension was shaken the higher was the resultant viscosity. The viscosity of the acidulated suspension was not, however, proportional to the degree of shaking. If it were identical, values for the constant b would be obtained regardless of the manipulation. The highest constant b was obtained for the series of suspensions which had been the most vigorously treated. These higher values may be due, as has been stated, to greater percentage of injury to proteins in dilute suspensions than in the more concentrated ones. Sufficient data are not available to justify drawing definite conclusions.

The resistivities of the 1000-cc. decantates of the flour suspensions extracted in different ways are found in Table IX. The more vigorous the treatment, the lower was the resistivity of the decantate. There was only a slight difference between the resistivities of the decantates in liter bottles filled with the flour suspension and shaken continuously and the decantates in those shaken at 10-minute intervals. There was, however, a considerable and significant difference between the resistivities of the decantates from the other two sets of bottles. The vigorous shaking, therefore, resulted in extraction of electrolytes sufficient markedly to affect the resistivity. As more electrolyte was removed, less is left in suspension to lower the viscosity when the suspension is acidulated.

Aside from the effect of the greater degree of mechanical manipulation in removing a greater quantity of electrolytes and consequently resulting in a greater viscosity of the acidulated suspension, the manipulation appeared to affect the viscosity in another way. This was made clear by a study of the viscosity and resistivity data (Tables VIII and IX) of the series of flours shaken continually and at 10-minute intervals for 7 hours. Altho there

was little difference in the resistivities of the decantates in these two series, the viscositity of the suspension treated the more vigorously during its preparation was greater in every case. For the 18-gram flour-water concentrations the resistivities of the decantates were the same, 854 ohms, while the viscosity of the suspension shaken continually for 7 hours was 216 degrees and that shaken at 10-minute intervals for the same time was 154 degrees. Analogous comparisons may be made with the other data in Tables VIII and IX. This phase of the subject will be left for further study.

The effect of different degrees of shaking on the protein content of the extract is given in Table X. It is evident that the protein decanted varies directly with the degree of shaking. Thus, the nitrogen content of the liter of decantate from the suspension maintained at 35° and shaken at intervals of 10 minutes for 45 minutes was 8.80 cg., while when the same conditions obtained except that half-filled bottles were shaken continually, the nitrogen content of the decantate increased to 12.05 cg. Hence more protein and more electrolytes are present in the decantate of vigorously shaken flour-water suspensions than in similar suspensions less vigorously shaken. The increase in electrolytes removed produces residues which are more electrolyte-free, hence greater viscosities result. The effect of the decanted protein in this regard is not known.

Since the electrolyte content of a suspension has so great an influence on viscosity, it appeared of interest to determine approximately how much electrolytic material was being decanted. Solutions of dibasic and monobasic potassium phosphate were considered as containing the ions most likely to be contained in the greatest concentration in decantates from flour suspensions. Solutions of these two salts were therefore prepared and their resistivities at different molarities were determined. The data obtained are given in Table XI and graphically in Figure 2. It is probable that KH_2PO_4 is the salt which occurs in highest concentration in flour extracts, as solutions of both this salt and of flour are on the acid side of pH 7.

The ash content of the flour used in this study was 0.44%. Eighteen grams of flour accordingly contained 0.0792 grams of ash. The resistivity of a decantate obtained from the flour extracted at 20° C. was 876 ohms. (See Table IV). Assuming that the electrolyte content of flour suspensions consisted entirely of KH_2PO_4 it is quite evident from Figure 2 and Table XI that the

resistivity of the 1000-cc. decantate (876 ohms) was equivalent in terms of KH_2PO_4 to approximately 50% more ash than was present in the original flour.

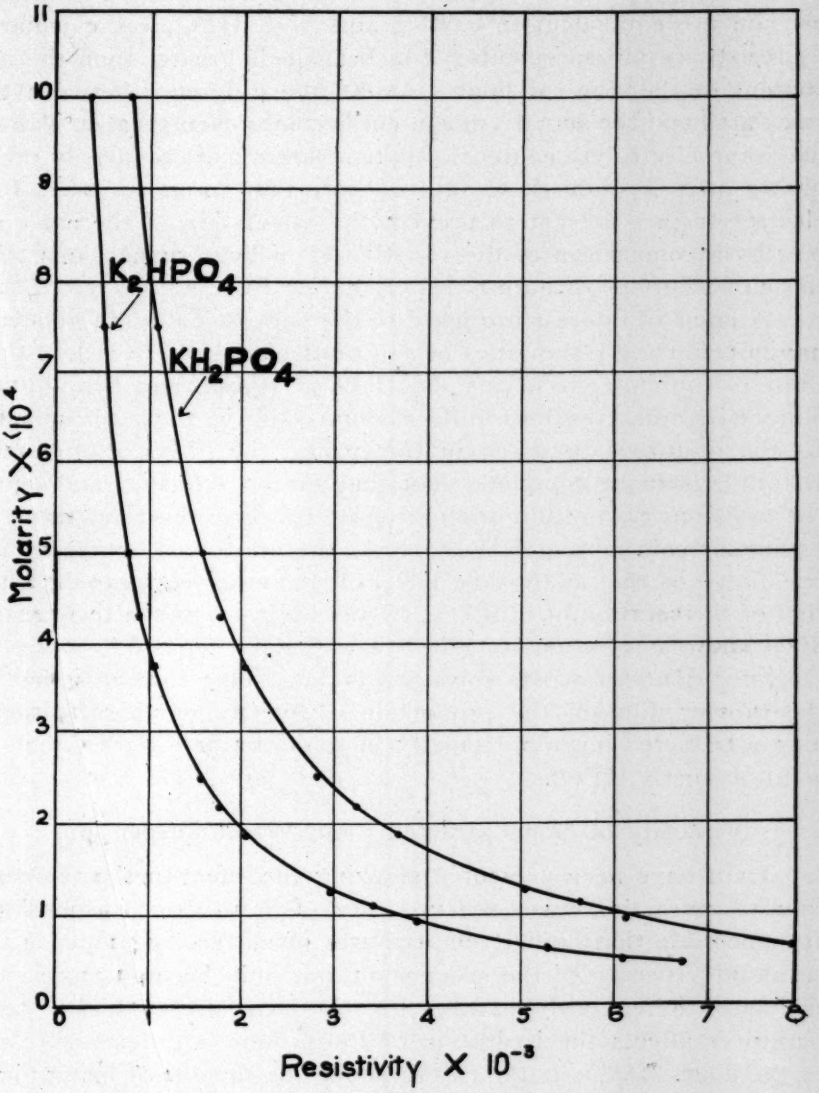

Fig. 2. Resistivities of Solutions of KH_2PO_4 and K_2HPO_4 at Different Molarities

Using similar data from Table IV and assuming that K_2HPO_4 is the only electrolyte in flour-water suspensions, it can be shown that the resistivity of the 1000-cc. flour-water decantate is equiva-

lent to the resistivity of 0.0773 grams of K_2HPO_4 dissolved in a liter of water. Following the same method of calculation, the additional 500-cc. of water removes electrolytes equivalent to 0.0067 grams of K_2HPO_4. Thus the total electrolytes removed from suspension are equivalent to 0.084 grams of K_2HPO_4 (as calculated by resistivity measurements), which again is greater than the ash content of the original flour. At 40° the difference between the calculated and the actual ash content becomes even greater. Hence while the electrolyte content of patent flours may be largely phosphates of potassium, these data indicate that enough of the other electrolytes are present to prevent the calculation of the ash content by a comparison of the resistivities of flour suspensions with those of solutions of pure K_2HPO_4 or KH_2PO_4.

A point of interest in regard to the data in Table XI and Figure 2 is that the resistivities of solutions of K_2HPO_4 are less than those of equimolar solutions of KH_2PO_4. Abbott and Bray (1909) obtained similar results for the sodium salts of these phosphates. At the dilutions discussed in this paper, the dissociation of the K_2HPO_4 is more complete than that of the KH_2PO_4 and hence the resistivities of solutions of K_2HPO_4 are lower than those of equimolar solutions of KH_2PO_4. As the dilution is increased the resistivity of the solution of KH_2PO_4 increases less rapidly than that of the solution of K_2HPO_4. Thus the ratio of the resistivities of 0.001 molar solutions of KH_2PO_4 and K_2HPO_4 is 2.1, while the ratio for 0.000054 molar solutions is 1.2. This indicates that at the greater dilution the proportion of swiftly moving hydrogen ions was increasing more rapidly in solutions of KH_2PO_4 than in solutions of K_2HPO_4.

Viscosity of Non-Extracted Flour-Water Suspensions

Data have been presented showing the effect of the temperature of extraction upon the viscosity of flour-water suspensions. It is possible that higher temperatures of extraction influence the resultant viscosity of the suspension, not only because more electrolytes are removed but also because exposure to higher temperatures affects the hydration of the protein aggregates present in the flour. If this is the case it should be capable of being made evident by determining the viscosity of non-extracted flour-water suspensions. Consequently the viscosities of non-extracted flour-water suspensions were determined. The flour-water concentrations were the same in this work as in the study on extracted suspensions. The proper quantity of flour in a 250-cc. Erlenmeyer

flask was shaken with about 85 cc. of distilled water at definite temperature and the flask was placed in a water bath at that temperature. The suspensions were maintained at temperatures which varied from 15° to 60° C. for an hour, being shaken at 10-minute intervals. At the end of an hour the suspension was made to 100 cc., the temperature of the suspension adjusted to 25° C., and the viscosity determined after acidulation with lactic acid. Syrupy lactic acid (instead of 20%) was used in order to obtain the maximum imbibition of the flour proteins.

The results are given in Table XII. It appears that the viscosity of flour-water suspensions is affected by temperature alone, but this effect is much less marked than that of electrolytes, especially at the higher flour-water concentrations. Thus at a concentration of 14 grams and a digestion temperature of 15° C. the viscosity was 10 degrees MacMichael, while under the same conditions but digested at 40° C. the viscosity increased to 20 degrees MacMichael. This is an increase in viscosity of 100%, which is almost equal to the percentage increase of extracted suspensions through the same temperature range. At a flour-water concentration of 20 grams, the viscosity of suspensions digested at 15° C. was 36 degrees MacMichael and when digested at 40° C. increased to 45 degrees. Thus the viscosity of the suspension prepared at the higher temperature was only 25% greater than that of the suspension prepared at 15°C. For the 16- and 18-gram flour-water concentrations, the percentage increase in viscosity over the temperature range of 15° to 40° C. is intermediate between 100 and 25%. In extracted flour-water suspensions, on the other hand, the percentage increase in viscosity over the same temperature range was practically constant regardless of concentration. This is indicated very clearly in the constants *b* for the extracted and non-extracted suspensions. They are nearly the same for the extracted suspensions, while they decrease quite regularly for the non-extracted suspensions.

The decrease in the value for *b* in the non-extracted flour-water suspensions suggests that certain substances are increasing in the suspensions of higher flour-water concentration, which results in a lowering of the viscosity. These substances might well be the greater quantity of electrolytes liberated by the increased activity of phytase at the higher temperatures. As the final volume of all the suspensions is 100 cc., it is obvious that those of higher flour-water concentration are also higher in electrolytes. If this reasoning is correct, increasing the range of flour-water concen-

tration ought to result in lowering the value for *b* more than in the studies reported in Table XII. Fifteen- and 24-gram samples of flour were therefore prepared according to the method given, and the viscosities of their suspensions were determined. The results are given in Table XIII. While some of the values for *b* are lower when the range in flour concentration is 9 grams than when it is 6 grams, other values are higher. The series of constants *b* in Table XIII, however, shows the same tendency to decrease with an increase in digestion temperature as does the series of constants listed in Table XII.

From the data given it is clear that the temperature to which a flour suspension has been exposed has a marked influence on its viscosity. Electrolytes in the non-extracted suspension, however, appear to mask the effect of temperature so that the viscosity of the non-extracted suspension is rather low as compared with that of the suspension from which the electrolytes have been partially removed. Other substances which lower viscosity may be present in the non-extracted suspensions also. Working (1924) has shown that the phosphatides may so operate.

Effect of Temperature on Hydration in Flour Suspensions

In Tables I and XII it may be noted that there is a fairly regular increase in viscosity in flour suspensions which have been exposed to temperatures varying from 15° to 40° C. In Table I it may also be noted that while the constant *b* was practically the same from 15° to 40° C., at 50° C. it increased abruptly and continued to increase at 55° and 60° C. Likewise the constant *a* had been increasing as the viscosity increased from 15° to 40° C. but at 50° it, also, changed suddenly from what its anticipated value should be (owing to increase in viscosity), because obviously the value for *b* had changed. All these sharp changes indicate that at 50° C. a change begins in the properties of the suspensions which had not been taking place at the lower temperatures. Data will be presented to indicate that this change is due to increased swelling of the constituents of the suspension at these higher temperatures.

Data in Table I show an increase in viscosity which is quite regular until a temperature of 50° is reached. Above this point there is a phenomenal increase in viscosity. The first time a temperature of 60° was used it was found impossible to reduce the volume of the residue to less than 160 cc. Viscosity determinations were accordingly made on the residues contained in 160 cc.

Even with 14, 16, 18, and 20 grams of flour in this quantity of water the viscosities were 130, 241, 440, and 583 degrees MacMichael, respectively. The constant *b* was 4.32. The experiment was repeated at the same temperature but the residues after decantation were centrifuged and additional water was decanted. By this method it was possible to obtain the residues in a volume of 100 cc. Before the addition of acid the viscosities of 16, 18, and 20 grams of flour in 100 cc. of water were 27, 55, and 97 degrees MacMichael, respectively. Upon the addition of acid, the viscosities increased to the high values shown in Table I, i.e., 380, 625, 1005, and 1810 degrees MacMichael for flour-water concentrations of 14, 16, 18, and 20 grams of flour per 100 cc. of suspension.[2]

The characteristics of the suspensions exhibiting these high viscosities were obviously different from those of the suspensions obtained at lower temperatures. When the acid was added to the suspension which had a viscosity of 1810 degrees MacMichael, it set as a solid gel. If stirred too vigorously it broke to form an irregular surface, but when stirred slowly a continuous surface was formed again. The gel had a bluish translucency which suggested starch gelantinization. If a beaker containing the gel were inverted it would not run out, even if inverted for several minutes. If the gel were allowed to stand for several hours or overnight it would synerise, a thin layer of liquid forming at the surface.

Some of the characteristics mentioned suggest that starch is beginning to play a part in the viscosity of the suspensions prepared at temperatures above 50° C. The data of Alsberg and Rask (1924) however, indicate that at 60° C. wheat starch has not begun to gelatinize sufficiently to affect the viscosity. These investigators state: "It will be noted that up to 65° C. there is no significant change in the viscosities of the suspensions. There is a slight decrease but this is probably due entirely to water the viscosity of which decreases similarly under these conditions. At 65° to 68° the viscosities begin to increase very gradually. As shown by curves there is also a uniform increase in the rate of increase of viscosity with respect to temperature until maximum viscosities are reached at 91° in cornstarch and 95° in wheat starch. The increases in viscosities are obviously due to simultaneous and therefore correspondingly gradual changes in the starch grains, changes which extend over a range of 25° to 30°."

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2 A No. 30 wire was used in the viscometer for most of the work reported. For these high viscosities, however, a No. 26 wire was used and the values were calculated to theoretical values for a No. 30 wire by the use of a factor.

Moreover the greatest change in viscosity does not occur until after the addition of the acid. Samec (1912, 1914) has shown that the addition of acids decreases the viscosity of pure starch pastes, hence it appears that the increase in viscosity upon the addition of acid is due to its effect on the proteins. Samec (1912) also noted that starch suspensions appeared more translucent even before changes in the individual granules could be observed under the microscope. Hence it is possible that changes in the starch granules may have occurred at 60° which it is impossible to measure by viscometric methods.

In order to determine, if possible, what changes took place when starch alone was exposed to room temperature and to a temperature of 60° C., 25-gram portions of household cornstarch were treated at these temperatures according to the methods used in preparing extracted flour-water suspensions for viscosity determinations. The viscosities of suspensions prepared at these two temperatures were determined with the MacMichael viscometer and were found to be the same (practically 0 degrees) regardless of the temperature of preparation. Other suspensions prepared at the same two temperatures were centrifuged at 525 × gravity for 10 minutes and the volumes of the material thrown down were determined. The volume occupied by the starch in the suspension prepared at room temperature was 24.5 cc., while that occupied by the starch in the suspension prepared at 60° was 45.8° C. From these observations it is apparent that the starch treated at 60° must have imbibed more water than that treated at room temperature. The appearance of the two suspensions before centrifugation was the same. The bluish translucency noted in flour-water suspensions prepared at 60° C. was not manifest in the starch suspension prepared at the same temperature.

The study using commercial starch indicated that at a temperature of 60° water is taken up by the starch and therefore less is left in the suspension as free water. As there is less free water, the addition of acid should result in a relatively greater increase in viscosity, since even more free water is removed. It will be recalled, however, that even when the volume of the suspension was 160 cc., the viscosities were markedly higher than those prepared at 40° when the volume of the suspension was 100 cc. The commercial starch maintained at 60° C. for 1 hour occupied a volume of 21.3 cc. more than that maintained at 25° C., and if the starch in the flour removed that quantity of water, the 60 cc. additional water should have been enough to compensate for it and

viscosities more nearly equal to those obtained at 40° should have resulted.[3] The difference between anticipated viscosity on the basis of water removed by starch hydration and actual viscosity, is great enough to warrant the suggestion that the explanation lies elsewhere; that the higher temperatures actually effect conditions which allow for a greater imbibition of water by the proteins when suspensions containing them are acidified; or that starch in suspensions containing protein does not behave in the same way as in pure starch suspensions.

In Table I are also given the viscosities of extracted flour-water suspensions prepared at 70° and 80° C. and at the boiling point. It may be noted that the viscosity of acidulated suspensions prepared at 70° and at 80° are lower than those of similar suspensions prepared at 60°. The viscosity of the suspension prepared at the boiling point is higher than that prepared at 80°, in spite of the fact that the viscosity in this case had to be determined on a suspension at a volume of 160 cc. The reason for this is that more complete gelatinization of the starch has undoubtedly taken place.

The data in Table I show that at a flour-water concentration of 16 grams of flour per 100 cc. of suspension, the viscosity of the acidulated suspension prepared at 60° C. was 625 degrees MacMichael; for a suspension prepared at 70° it decreased to 465 degrees; for one prepared at 80° it decreased further to 154 degrees; and for one prepared at the boiling point (of different flour-water ratio, however), it increased to 196 degrees. In the same suspensions the viscosities before acidulation were: at 50° C., 2 degrees MacMichael; at 60° C., 27 degrees; at 70° C., 120 degrees; at 96° C., 145 degrees. Thus, above 60° viscosities before acidulation increase while those after acidulation decrease. This indicates that as the suspensions were exposed to higher temperatures during their preparation, the effect of the temperature on the starch was such as to favor imbibition, while exposure to temperatures above 60° C. had just the opposite effect on proteins as determined when suspensions containing them were acidulated. In one instance 16 grams of flour suspended in a liter of water was boiled for 6 hours. As much of the water as possible was decanted and the residue was washed with an additional 500 cc. of water. After the decantation of the 500 cc. of water, the residue was made up to 160 cc. and the viscosity determined. The viscosity of the suspension

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[3] The viscosities obtained when 100-cc. and 160-cc. suspensions were used are not strictly comparable, as the total volumes were used for the viscosity determinations in each case. It is believed, however, that the viscosity at the greater dilution is different enough from what might be anticipated from dilution alone that comparison is possible.

before acidulation was 270 degrees MacMichael. After acidulation it increased to 305 degrees. Under this treatment it appears that the viscosity before acidulation is high while the increase due to acidulation is only slight as compared with such increases determined when the suspension was exposed to lower temperatures.

It was thought that by boiling a flour-water suspension all the starch would be gelatinized, and since such a low ratio of flour to water (16 to 1000) was used, all the starch could be removed by decantation and the viscosity of the relatively starch-free protein determined. Such was not the case, however, for when flour-water suspensions were boiled even as long as 6 hours a precipitate could be obtained, either by centrifuging or by allowing it to stand, which was by no means starch-free. The weight of the dry precipitate was equivalent to about half the weight of the original flour. The protein content of precipitates prepared by boiling 16 grams of flour in a liter of water for 1 or for 2 hours and then leaching with 500 cc. of water at room temperature was 13.38% and 13.68%. The protein content of this original flour was 12.37%. This indicates that starch and protein were removed in almost the same proportion when the flour-water suspensions were boiled.

In order to investigate further the effects of exposing starch and protein (as flour) to a temperature of 60° C. and to determine, if possible, the viscosity of the protein in suspension separate from that of the starch, other methods were sought. If all the starch could be removed by its conversion to soluble sugars by enzymes, the resultant viscosity of the acidulated suspension should be due to the protein which remained. The difficulty encountered in following such a procedure, however, lies in the uncertainty of obtaining a diastase which is non-proteolytic. For this reason this line of experimentation was not attempted. The other line of attack was obviously to remove the protein by a protease and determine the viscosity of the resultant starch suspension. Any marked effect of the protease preparation on the starch could be determined by gelatinizing at a higher temperature the starch present in the suspension after treatment with the protease. In following out this idea, four 18-gram portions of flour suspended in 1000 cc. water were digested at 40° C. over night. One cc. of toluene was added to each suspension as a preservative, and to two of them was added 0.25 gram of Mercks pepsin. At the end of the digestion period one preparation containing pepsin and one

without pepsin were extracted with an additional 1000 cc. of water at 40°. After the decantation of as much liquid as possible, the flour residues were made to 100 cc., cooled to 25° C., acidulated, and the viscosities determined. The viscosity of the suspension digested with protease was 0 degrees MacMichael, while that of the suspension digested without protease was 160 degrees. The supernatant liquid from the other set of flasks was decanted and an additional liter of water at 60° C. was added. The two suspensions were maintained at that temperature for 1 hour, being shaken at 10-minute intervals. At the end of this time and allowing time for the flour to settle to the bottom of the flask, most of the liquid was again decanted and the suspension made to 100 cc. The suspensions were cooled to 25° C. and the viscosities before and after acidulation were determined. The viscosity of the suspension treated with pepsin was 0 degrees MacMichael in both cases. The viscosity of the suspension not treated with pepsin was 77 degrees MacMichael before acidulation. As these great changes in viscosity were known to occur upon the acidulation of such suspensions, the volume was made to 160 cc., whereupon the viscosity decreased to 15 degrees. Upon acidulation, the viscosity increased to 550 degrees MacMichael. As the pepsin resulted in a reduction of the viscosity to 0 degrees MacMichael regardless of the temperature to which the suspension was exposed, and as exposure of the suspension not containing proteoclastic enzymes to a temperature of 60° C. resulted in producing such high viscosities, it is believed that the sudden change in viscosity which occurs at this temperature is due almost entirely to the effect on the protein. That the starch was not affected to any great extent by the enzyme preparation or by exposure to the temperature of 60° C., is indicated by the fact that suspensions obtained by treatment with the enzyme over night and by similar treatment plus exposure to a temperature of 60° C. (both suspensions having a viscosity of 0 degrees MacMichael) set as solid gels when they were heated to 96° C. This was obviously due to a gelatinization of the starches.

As the temperature to which a commercial starch suspension was exposed appeared to affect the volume occupied by the precipitate after centrifugation, it was considered of interest to make similar determinations on flour-water suspensions exposed to temperatures up to 60°. Accordingly, 25-gram samples of flour were weighed into bottles of about 125 cc. capacity and enough distilled water was added to make the volume of the suspension 100 cc.

After being thoroly shaken, the bottles were placed in water baths maintained at temperatures of 20°, 30°, 40°, 50°, 55°, and 60° C. They were allowed to remain in the water baths for 1 hour, being shaken at 10-minute intervals. At the end of the hour they were removed from the water baths and allowed to stand perfectly still for 1 hour. At the end of the second hour the volume occupied by the flour which had settled out was noted. The bottles were then centrifuged at 525 × gravity for 10 minutes, and the volumes occupied by the precipitates were determined. The results are given in Table XIV.

From the data presented in Table XIV it may be noted that between 20° and 40° C. there is no significant difference in the volumes occupied by the rich-in-flour phase of the suspensions whether the volumes were determined by letting stand for 1 hour or by centrifuging. At 50° C., however, the rich-in-flour phase begins to show a tendency to occupy a relatively greater volume. This property becomes more marked at 55° and 60°. It is significant that the property of the rich-in-flour phase to occupy a greater volume appears at the same point that viscosity, constant *b*, and constant *a* change suddenly from anticipated values. The physical chemistry of the changes which occur at this point will bear further investigation. It is possible that the temperature at which the sudden change occurs will be different for different flours and that this may be of significance in relation to flour strength. These considerations, however, will be left for future study.

In Table XII it may be noted that for the viscosity determinations at 60° C., two sets of observations appear which are quite different. These determinations were made under what were considered to be identical conditions. It was recalled, however, that in preparing the first series of suspensions for their 1 hour of digestion, less water was used than when the second series was prepared. Hence, when the suspensions were made to 100 cc. (immediately before the viscosities were determined) more water had to be added to the suspensions in the first series than to those in the second series. It was possible that the water added immediately before the viscosity was determined was not held in the same manner as that which had been present throughout the whole digestion period. As the suspensions listed first in Table XII had more of the recently added water and hence, we will assume, had more water which had not had time to enter so intimately into the structure of the colloid, there would be a possibility of this series displaying lower viscosities.

This hypothesis was tested out in two ways and is believed to be correct. Twenty-gram portions of flour were suspended in 85 cc. and 70 cc. of water at 60° C. These suspensions were maintained at 60° for an hour, being shaken at 10-minute intervals. At the end of the hour sufficient water was added to make 100 cc. and after thoro shaking the bottles were centrifuged at once. The data are given in Table XV. The centrifugate of the suspension digested with 70 cc. of water occupied a volume of 60.4 cc., while that digested with 85 cc. of water occupied 71.5 cc. This indicates that 11 cc. more of the water is held colloidally in the suspension digested at the lower flour-water ratio than in the suspension digested at the higher flour-water ratio. This water is so firmly held in the colloid structure that 10 minutes of centrifuging at 525 × gravity is unable to remove it.

It was thought that if given sufficient time the recently added water would be taken into the colloidal structure to the same extent as water which had been present throughout the digestion period. Consequently, after the digestion period of 1 hour two suspensions identical with the two already discussed were made to a volume of 100 cc. and allowed to stand in the laboratory for another hour. At the end of this period the volumes of flour which had settled out were noted and the bottles containing the suspensions centrifuged. The data obtained are also given in Table XV. These data indicate that additional water is taken into the rich-in-flour phase but it occurs to much greater extent in the suspension which already had more water in its rich-in-flour phase. Thus the centrifugate of the 20-gram suspension digested with 70 cc. of water at 60° C. for 1 hour occupied a volume of 60.4 cc. when centrifuged immediately after digestion, while the volume occupied by the centrifugate increased to 65.5 cc. when the suspension was made to 100 cc. and allowed to stand at room temperature for 1 hour after digestion. The centrifugate of a similar suspension digested with 85 cc. of water had a volume of 71.5 cc. when centrifuged immediately after digestion. When a similar suspension was made up to 100 cc. and allowed to stand at room temperature for 1 hour, the volume occupied by the centrifugate increased to 82.7 cc. The data in regard to the volume to which the flour would settle when allowed to stand are indicative of the same phenomenon.

In order to carry this study one step further, suspensions of different flour-water ratios were digested for 1 hour at 60°. At the end of the digestion period the volumes were made to 100 cc. and the viscosities determined. Samples of 15 and 20 grams of flour

were digested with 85 and 70 cc. of water. The results are given in Table XVI. The data indicate that the lower the flour-water ratios at which the digestion is made, the higher is the viscosity of the resultant suspension made to a volume of 100 cc. Thus, when the digestion was made with 85 cc. of water and 20 grams of flour, the viscosity of the acidulated suspension was 367 degrees MacMichael. Under identical conditions, except that 70 cc. of water was used during the digestion period, the viscosity was 217 degrees. The data in Table XVI show similar results when the viscosities of unacidulated suspensions are compared. A probable mechanism for this phenomenon has already been discussed.

Relation of Proteolytic Activity to Viscosity

Before change in the viscosity of flour suspensions can be used as an index of proteolytic activity, the factors which affect the absolute viscosity of flour suspensions must be understood. From the preceding work it appears that electrolyte content and degree of hydration of the constituents of the suspension influence viscosity to the greatest degree. Both these factors are functions of the temperature: the electrolyte content, because at higher temperatures more electrolytes are rendered available for removal by decantation; hydration, because at higher temperatures different degrees of hydration of starch and protein occur. Electrolyte content is also a function of time. Longer digestion periods permit more electrolytes to be formed by phytase-phytin phenomena, hence such electrolytes may be decanted. Hydration is also a function of time, but after 1 hour and before 24 hours the writer does not consider it an important factor. Below a temperature of 50° C., moreover, hydration does not appear to play so important a part.

On the basis of these observations it appears that the initial viscosity of a flour suspension, the proteolytic activity of which is being studied, should be determined under such conditions as permit of obtaining the highest value possible, assuming complete removal of electrolytes and exposure to conditions permitting hydration of the flour constituents comparable to the conditions responsible for this phenomenon when flour suspensions are autodigested. It is believed that the closest approach to these conditions is attained when the viscosity is determined on suspensions which have been extracted at 40° C. with 1 liter and 500 cc. successively, or a slight modification of this procedure, as will appear later. Under these conditions the greatest quantity of electrolytes

is removed in the smallest possible volume of water. With this shortest period of extraction, moreover, extreme hydration of the flour constituents does not take place at this temperature.

The data in Table XVII illustrate some of the points mentioned. When 20 grams of flour were extracted with water at 40° C. in the usual way (1000 cc. of water for 1 hour + 500 cc. of water for 15 minutes) a viscosity of 257 was obtained. When extracted with an additional 500 cc. of water for an hour the viscosity increased only 10 degrees. When the suspension extracted at 40° in the usual way was kept at low temperature for an hour after extraction, the viscosity increased to 272 degrees MacMichael. When the treatment was the same except that the suspension kept at low temperature was extracted with 500 cc. of water, the viscosity increased further to 297 degrees MacMichael. When flour suspensions were extracted in the regular way at 20° C. a viscosity of 134 degrees MacMichael was obtained. When suspensions similar to the preceding were held at 20° for 1 hour after the final decantation, the viscosity increased to 168 degrees. Since no electrolytes were decanted, this indicates that hydration was going on. The hydration was greater at 20° than at 40° at which temperature, under the same conditions, the viscosity only increased 10 degrees MacMichael when the suspension was held over for 1 hour before making the viscosity determinations. Another suspension was extracted at 20° in the usual way and then kept at 40° for 1 hour before making the viscosity determination. In this case the viscosity fell to 122 degrees, indicating that the quantity of electrolytes in the suspension increased to such an extent that a lower viscosity was obtained. Still another suspension was extracted at 20° C. in the usual way and then extracted with an additional liter of water at 40°. This viscosity increased to 310 degrees MacMichael. This indicates that the additional extraction of this suspension at 40° removed even more electrolytes than were removed by three extractions at 40° C. It is quite possible that this is due to a relatively high rate of enzyme action on the relatively small quantity of substrate remaining when the liter of water at 40° was added. When the extraction temperature is 40° during the whole period of extraction, the rate of phytase activity is rapid at first but slows up toward the end of the extraction time. These data indicate that with extraction at 40° C. the viscosities obtained are nearer the true maximum values than the values obtained at lower temperatures.

The resistivity data given in Table XVII show very clearly what is happening in regard to the electrolytes. In treatment 1, the resistivity of the 500-cc. decantate was 4178 ohms. After the residue was centrifuged the resistivity was found to be 3151 ohms, a value considerably lower than the resistivity of the extract. This, however, might have been anticipated. When the residue was maintained at 40° for 1 hour after the last decantation, the resistivity in the decantate after centrifuging decreased to 2984 ohms. This might also be anticipated, since more electrolytes would be elaborated during the additional hour. The resistivity of the decantate after centrifuging shows a considerable difference in treatment 3. This abnormality will be investigated further in another section.

The difference between the resistivity of the decantate after centrifuging and that of the 500-cc. decantate shown in treatment 5, is greater than is the difference between the two observed in the case of treatment 1. As the time after decantation is increased or if the suspension is exposed to a higher temperature, the resistivity of the decantate after centrifuging decreases. Thus, the resistivity of the 500-cc. decantate was 3958 ohms; that of the decantate obtained by centrifuging immediately after decantation was 2481 ohms; that of the decantate from the suspension centrifuged after 1 hour at 20° was 1542 ohms, and that of the suspension centrifuged after 1 hour at 40° was 904 ohms. When the original extraction temperature was 40° C. the changes in resistivities due to similar manipulations were relatively slight. Hence, it is evident that suspensions extracted at lower temperatures contain considerable quantities of electrolytes or systems capable of elaborating them.

The resistivities (Table XVII) obtained when flour-water suspensions extracted at 40° were cooled, indicate that temperature may also be associated with the degree of adsorption of some of the electrolytes present in suspensions. Thus the resistivity of the 500-cc. decantate reported in treatment 3 was 4084 ohms. When the residue was cooled to 6° C. and centrifuged after an hour at this temperature, the resistivity increased to 4995 ohms. The same increase was obtained in several instances when suspensions similarly prepared were allowed to stand for 24 hours.

In order to investigate this point further, the series of experiments given in Table XVIII was carried out. In this study the resistivities of the 1000-cc. decantates from flour-water suspensions extracted at 20° and at 40° C. were compared with those from the centrifuged residues treated in different ways. The liter decantate

from a suspension extracted at 40° had a resistivity of 757 ohms. Heating or cooling had no significant effect. The resistivity of the decantate from the residue after centrifuging, however, was 694 ohms—considerably lower than that of the liter decantates. This indicates that while the suspension is settling, before decantation, electrolytes are being elaborated in the rich-in-flour phase of the suspension. When the suspension was cooled to 5° C. before centrifuging, however, the resistivity of the decantate increased to 829 ohms. This indicates that the cooling resulted in greater absorption of electrolytes by the flour colloids.

The same phenomenon occurred when the temperature of extraction was 20° C., but to a lesser degree. Thus, the resistivity of a 1000-cc. decantate obtained at 20° C. was 788 ohms. The resistivity of the decantate obtained from the residue after centrifuging was 703 ohms, while that obtained when the cooled residue was centrifuged was 753 ohms.

In Tables XIX and XX are given the results obtained when flour-water suspensions were digested for 24 hours at two temperatures and under two sets of conditions. One cc. of toluene was added to the suspensions to prevent bacterial action. The data in Table XIX were obtained by allowing 18 grams of flour suspended in 1 liter of water to digest for 24 hours at temperatures of 20° and 40° C. At the end of this time the flour suspensions were washed with varying quantities of water and the viscosities determined. The maximum viscosity was 208 degrees MacMichael for the suspension digested at 20°, and 186 degrees for that digested at 40° C. Data in Table VI show that when 18-gram portions of flour were extracted with varying quantities of water at 40° C., the maximum viscosity attained was 224 degrees. In connection with the data in Table XIX, it appears that if the true viscosity of the flour were 224 degrees MacMichael, proteases are responsible for a decrease in viscosity to 208 degrees when the suspension is digested for 24 hours at 20° C. and to a viscosity of 186 degrees when the temperature is 40° C.

In obtaining the data given in Table XX the procedure was so modified that the suspensions were extracted for one hour with 1 liter of water at 40° C. and then 1 liter, or 500 cc., of additional water at 6° or at 40° C., was added to the residue and the digestion allowed to proceed at the two temperatures for 24 hours. The temperature of 6° was maintained by placing the flasks in a sink and allowing cool tap water to run over them. It was thought that at this low temperature the rate of proteolytic activity would be very

low and a better idea might be gained of the maximum hydration capacity of the flour and its effect on viscosity. The maximum viscosity obtained at this low temperature was 233 degrees MacMichael, a value only slightly above that exhibited by the same quantity of flour extracted at 40° C. with varying quantities. This indicates that flour in suspension attains practically as great hydration upon extraction with several liters of water at 40°C. as when it is extracted with 1 liter and then given 24 hours at low temperature to hydrate.

The maximum viscosity attained by the suspension extracted with 1 liter of water at 40° and then digested for 24 hours at the same temperature was 203 degrees MacMichael. This viscosity was exhibited by the suspension extracted with 4 liters of water. The lower viscosity in this instance indicates also the action of proteases.

A comparison of the data in Tables XIX and XX indicates that proteases or materials accelerating their activity are decanted with the first liter of decantate. Thus, in the case of the suspensions digested at 40°, the suspension which was extracted after 1 hour of digestion showed a maximum viscosity of 203 degrees MacMichael after 24 hours, while the maximum viscosity of the suspension which was not decanted until after 24 hours was 186 degrees.

Discussion

Before changes in the viscosity of flour-water suspensions could be used as an index of proteolysis or the rate of proteolysis, it was necessary to investigate the factors which affect the viscosity of flour suspensions. When extracted flour-water suspensions were used in the determination of the viscosity, time and temperature of extraction appeared to be the factors affecting to the greatest degree the final viscosity. The temperature of extraction, keeping the time constant, markedly affects the viscosity of the suspensions, viscosity increasing as the temperature of extractions is raised. The increase in viscosity may be due (1) to the removal of greater quantities of electrolytes at the higher temperatures of extraction, (2) to the removal of greater quantities of protein associated with a lower viscosity, and (3) to greater hydration of the proteins responsible for viscosity at the higher temperature. The data indicate that suspensions extracted at higher temperatures are susceptible to the effects of these factors, the degree increasing with the temperature. From 15° to 40° C. the effect of these factors is a constant at any temperature, regardless of the flour-water

concentration. This is indicated by the fact that the constants *b* are the same throughout this temperature range. At 50° and above, changes occur which result in sudden changes of the constants *b*. This indicates that the changes are not of the same degree at all flour-water concentrations. Sudden changes in the degree of hydration of starch and protein at these temperatures seem to be responsible for the results; hence the relative quantity of free water in the suspension decreases considerably at 50° C. and above. This explanation may account for the enormous changes in viscosity which occur at 60° C. Below 50° the effect of temperature on degree of hydration is apparently less, the different temperatures affecting approximately the same degree of hydration. It is suggested that the temperature at which the sudden changes in constant *b* and viscosity occur may be different for different flours and hence may be worthy of investigation in accounting for difference in flour strength.

Time of extraction also influences the final viscosity of a flour-water suspension, since longer periods of extraction result in the removal of greater quantities of electrolytes and also allow for greater hydration of the proteins responsible for viscosity. No data were obtained on the effect of time of extraction on the quantity of protein removed. It is obvious that the time of extraction should not be long enough to allow proteases to act sufficiently to lower viscosity.

In regard to the effects of time and temperature on degree of hydration, it may be noted that by no manipulation at a lower temperature could the final viscosity be brought to an equal value with that obtained with similar manipulation at a higher temperature. Instances of this occur in the data in Tables IV and VI. The maximum viscosity obtained at 20° approaches but does not attain that obtained at 40°. Thus, the data in Table IV show the viscosities determined after 1 hour of extraction at 20° and at 40° to be 110 and 189 degrees MacMichael, respectively, while the maximum viscosities attained at these two temperatures by prolonging the time of digestion were 162 and 205 degrees. As the resistivities of the decantates from the flour extracted at 20° and digested the longest time were so nearly equal to those from the flour extracted at 40°, it would be expected that their viscosities should be more nearly the same. As this was not the case, some factor other than electrolytes in the suspension must operate to increase the viscosity of the suspension exposed to a temperature of 40° C. Data such as these furnish evidence for attributing significance to the effect

of hydration on the viscosity of flour-water suspensions. The data in Table XIX, moreover, indicate that if suspensions exposed to lower temperatures are given sufficient time their viscosities might so increase as to become equal to those obtained for suspensions exposed to higher temperatures, provided there were no proteolytic activity during this time. In Table XIX the maximum viscosity attained after 24 hours by suspensions exposed to a temperature of 20° C. was 203 degrees MacMichael, while that attained by suspensions exposed to 40° C. was 186 degrees. The maximum viscosity attained by a similar suspension exposed to 40° C. for a short time was 224 degrees. Hence, assuming no proteolytic activity, it is quite possible that the suspension maintained at 20° for 24 hours might have attained a viscosity of 224 degrees.

In determining the proteolytic activity of a flour by viscometric methods, the factors which have been mentioned must be considered. It appears that the extraction of a suspension with one liter of water at 40° C. followed by an extraction with an additional 500 cc. of water, removed practically all the electrolytes that can be removed. At 50° and 60° C. a slightly greater quantity of electrolytes is removed, owing probably, to the greater solubility of some of the electrolytes of the flour or a lower absorption of them by the flour, than to a greater quantity of electrolytes being elaborated by the action of phytase on phytin. The data in Table XVIII indicate the effect of temperature on absorption of electrolytes by flour particles in suspension. Exposure of the flour to a temperature of 40° C., moreover, does not effect the relatively tremendous changes in hydration capacity which are effected at higher temperatures. In comparing a series of viscosity determinations made on the same flour-water concentrations but digested for different periods, it is desirable to remove the same quantity of electrolytes and to effect the same degree of hydration. It has been shown that longer periods of digestion at lower temperatures result in the removal of practically the same quantities of electrolytes as short periods of digestion at high temperatures. It appears, also, that the degree of hydration of flour in suspensions exposed to low temperatures for long periods of time may attain that of flour in suspensions exposed to higher temperatures (under 40° C.) for short periods of time. For these reasons, in order that the viscosities of flour-water suspensions digested for different periods of time may be strictly comparable, the suspension should be exposed to the same conditions so far as the removal of electrolytes and hydration of the flour constituents is concerned. This

means that each suspension should be treated in a method comparable to extraction with water at 40°, according to the method of Gortner, or better, extracting with several liters of water at 40° C. until a maximum viscosity is obtained.

A single flour has been used for all the work reported in this paper. While it is probable that other flours will vary in the degree in which they change when suspensions in water are treated according to methods comparable with those used, it is believed that the principles involved will be the same. The flour was more than a year old, however, and as the work of Stockham (1920) and others suggested that the proteolytic activity of flours may decrease with age, this flour may show results different from flours more recently milled.

Summary

The temperature at which flour-water suspensions are extracted in preparing them for viscosity determinations affects the quantity of electrolytes removed, the quantity of protein removed, and the degree of hydration of the flour in suspension; the higher temperatures increasing the quantity of electrolytes and protein removed and also the degree of hydration of the flour.

Increasing the time of extraction at lower temperatures tends to effect the same results as exposure to higher temperatures for shorter periods of time, i. e., more complete removal of electrolytes and greater degree of hydration.

Degree of manipulation affects the quantity of electrolytes and proteins removed from a flour-water suspension. The more vigorously a suspension is shaken, the greater the quantity of these materials removed.

Viscosity depends on the quantity of electrolytes present in the suspension the viscosity of which is being determined, and the degree of hydration of the flour constituents of the suspension. Other factors operate, also, but these two were investigated.

Degree of hydration of the flour in suspension changes suddenly at 50° C.

Constants b which are identical may be obtained if the temperature at which the series of viscosity determinations are made is the same and if the determinations are made at temperatures not higher than 40° C.

Constants a which vary as the logarithm of the factor for converting the viscosity at one temperature to that at a higher temperature, may be obtained if the constants b are the same.

Rate of proteolytic activity of a flour suspension can be measured by viscometric methods only if the degree of hydration of the suspended flour and the quantity of electrolytes present in the suspension can be made constant when the viscosities of several suspensions digested for different periods of time are determined.

Comparable conditions in regard to electrolytes and degree of hydration are attained when extractions are made at 40° C. with several liters of distilled water.

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Appendix

TABLE I

VISCOSITIES OF FLOUR-WATER SUSPENSIONS FROM WHICH THE ELECTROLYTES HAVE BEEN EXTRACTED AT DIFFERENT TEMPERATURES BY WASHING WITH 1000 CC. OF WATER FOR ONE HOUR AND 500 CC. OF WATER FOR 15 MINUTES

Temperature °C.	Viscosity: Flour-water concentration gm. per 100 cc. 14	16	18	20	Constant b	Constant a
	°MacM.	°MacM.	°MacM.	°MacM.		
15	39	61	97	119	3.14	−1.99
20	45	73	104	139	3.15	−1.95
25	54	80	116	162	3.08	−1.81
30	69	106	154	210	3.12	−1.74
35	76	115	167	230	3.11	−1.68
40	82	122	173	255	3.15	−1.70
50	89	149	226	326	3.63	−2.21
55	140	265	410	585	3.98	−2.41
60	380	625	1005	1810	4.32	−2.39
70	265	465	....	....	4.21	−0.073
80	86	154	....	....	4.36	−0.093
96	....	196	....	....	....	....

TABLE II

TEMPERATURE COEFFICIENTS THROUGH TEMPERATURE RANGES GIVEN IN FIRST COLUMN

These calculations are based on the data in Table I.

Temperature range °C.	Calculated coefficient per °C. Flour-water concentration, gm. per 100 cc. 14	16	18	20	Average coefficient per °C. based on data obtained from four different flour-water concentrations
15 to 20	1.032	1.040	1.028	1.034	1.033
20 to 25	1.040	1.018	1.022	1.032	1.028
25 to 30	1.054	1.064	1.064	1.064	1.061
30 to 35	1.020	1.016	1.016	1.018	1.017
35 to 40	1.014	1.012	1.006	1.020	1.013
40 to 50	1.008	1.022	1.031	1.028	1.022
50 to 55	1.124	1.142	1.162	1.158	1.144
55 to 60	1.342	1.270	1.294	1.418	1.331
20 to 40	1.041	1.033	1.033	1.041	1.037

TABLE III

RESISTIVITY OF DECANTATES FROM FLOUR-WATER SUSPENSIONS

Resistivities were determined immediately after decantation and 168 hours later on the same decantate Resistivity at 28°C.

Temperature	Fresh decantate: Flour-water concentration 14	16	18	20	168 Hours after decantation: Flour-water concentration 14	16	18	20
°C.	ohms	ohms	ohms	ohms	ohms	ohms	ohms	ohms
15	1140	1039	917	848	1121	1008	907	807
20	1121	989	885	801	1093	936	857	782
25	1077	936	867	788	1061	838	826	782
30	1052	951	854	782	1052	936	845	772
35	1049	876	835	768	1052	819	807	710
40	1033	911	838	760	1027	911	829	744
50	1046	920	816	750	1033	917	823	753
60	1021	898	810	728	1014	882	725	713
80	1058	920	...	...	1046	914	...	...

TABLE IV

VISCOSITY OF FLOUR SUSPENSIONS AND RESISTIVITY OF THEIR EXTRACTS WHEN SUSPENSIONS WERE EXTRACTED WITH THE SAME WATER AT 20° OR AT 40°C. FOR DIFFERENT PERIODS OF TIME

Eighteen-gram portions of flour were used.

Time in contact with 1 liter of water	Viscosity		Resistivity at 28°C.			
	Temp. of wash water at 20°C.	Temp. of wash water at 40°C.	1000 cc. of decantate obtained at 20°C.	500 cc. of decantate obtained at 20°C.	1000 cc. of decantate obtained at 40°C.	500 cc. of decantate obtained at 40°C.
hr.	°MacM.	°MacM.	ohms	ohms	ohms	ohms
1	110	189	876	4640	820	4900
2	156	205	845	4900	815	4900
3	152	192	833	4712	808	4618
4	154	172	824	5152	812	4963
5	162	184	824	4900	815	4900
6	150	179	832	5246	809	....
7	151	171	819	5434	806	4775
8*	148	...	825	5654	...	....

*Placed in a water bath at 40° for the last two hours.

TABLE V

VISCOSITY OF FLOUR SUSPENSIONS AND RESISTIVITY OF THEIR EXTRACTS WHEN SUSPENSIONS WERE EXTRACTED WITH THE SAME WATER AT DIFFERENT TEMPERATURES FOR VARYING PERIODS OF TIME

Twenty-gram portions of flour were used.

Temperature	Viscosity of flour suspensions in contact with 1 liter of water for various periods of time, washed with 500 cc. of water				Resistivity of 1000 cc. of decantate from flour-water suspensions			
	1 hr.	2 hr.	3 hr.	4 hr.	1 hr.	2 hr.	3 hr.	4 hr.
°C.	°MacM.	°MacM.	°MacM.	°MacM.	ohms	ohms	ohms	ohms
15	120	160	187	209	852	826	807	791
20	142	200	215	216	829	788	785	779
25	165	218	234	233	794	779	785	755
30	211	263	240	269	786	768	749	749
35	230	277	278	277	768	750	739	741
40	255	242	280	280	757	739	738	734
50	330	365	335	325	749	726	722	722

TABLE VI

VISCOSITY OF FLOUR-WATER SUSPENSIONS EXTRACTED WITH DIFFERENT QUANTITIES OF WATER

Eighteen-gram samples of flour were used.

Wash water used	Viscosity	
	Maintained at 20° during extraction of electrolytes	Maintained at 40° during extraction of electrolytes
Liters	°MacM.	°MacM.
1	101	160
2	116	199
3	130	222
4	130	216
5	147	218
6	157	224
7	169	189
8	173	207
9	180	....
10	174	....
11	170	....

TABLE VII

EFFECT OF TEMPERATURE AT WHICH ELECTROLYTES ARE EXTRACTED FROM A FLOUR-WATER SUSPENSION ON QUANTITY OF PROTEIN EXTRACTED*

	Nitrogen extracted							
	Flour-water concentration							
Temp.	14	16	18	20	14	16	18	20
°C.	cg.	cg.	cg.	cg.	%	%	%	%
15	7.56	7.86	8.76	9.56	24.8	22.6	22.4	22.0
20	7.66	8.21	9.31	9.71	25.1	23.6	23.0	22.3
35	9.41	10.42	10.70	11.35	30.9	30.0	27.6	26.1
50	10.00	11.27	12.33	13.53	32.9	32.4	31.3	31.1
55	10.86	11.16	13.38	15.35	35.6	32.1	34.2	35.3
60	11.62	12.73	13.63	15.00	38.2	36.6	34.8	34.5
70	11.95	14.49	....	....	39.3	41.7	....	....
80	11.09	12.54	....	....	36.4	36.1	....	....
96	9.41	10.66	....	....	30.9	30.7	....	....
96	....	7.39	....	....	....	21.2	....	....

*The original nitrogen content of the flour was 2.17 per cent.

TABLE VIII

EFFECT OF METHOD OF EXTRACTING ELECTROLYTES FROM FLOUR SUSPENSION ON VISCOSITY OF THE ACIDULATED SUSPENSION*

Method of extraction	Flour concentration				Constant *b*
	14	16	18	20	
	°MacM.	°MacM.	°MacM.	°MacM.	
	Temperature of extraction, 35°C.				
Continuous shaking for 45 minutes, 1000 cc. of flour suspension in a 2000 cc. flask.	90	140	205	294	3.31
Continuous shaking for 45 minutes, 1000 cc. of flour suspension in a 1000 cc. flask.	80	123	180	236	3.06
Shaken at 10-minute intervals for 45 minutes.	76	115	167	230	3.11
	Temperature of extraction, 20°C.				
Continuous shaking for 45 minutes, 1000 cc. of flour suspension in a 2000 cc. flask.	51	84	127	175	3.47
Continuous shaking for 45 minutes, 1000 cc. of flour suspension in a 1000 cc. flask.	49	80	114	148	3.10
Flask shaken at 10-minute intervals for 45 minutes.	45	73	104	139	3.15
Continuous shaking for 7 hours, 1000 cc. of the flour suspension in a 2000 cc. flask.	85	146	216	306	3.60
Flask shaken at 10-minute intervals for 7 hours.	65	104	154	212	3.39

*The first extraction was made with 1000 cc. of water and the final extraction with 500 cc. The flour residue was made to 100 cc. before acidulation, i.e., before viscosity was determined.

TABLE IX

RESISTIVITIES OF FIRST DECANTATE OBTAINED WHEN FLOUR-WATER SUSPENSIONS ARE EXTRACTED WITH 1000 CC. AND 500 CC. OF WATER ACCORDING TO THE METHODS OF EXTRACTION GIVEN

Method of extraction	Flour concentration			
	14	16	18	20
	ohms	ohms	ohms	ohms
		Temperature, 35°		
Continuous shaking for 45 min., 1000 cc. of a suspension in a 2000 cc. flask.	1061	939	854	785
Continuous shaking for 45 min., 1000 cc. in 1000 cc. flask.	1099	986	885	813
Flask shaken at 10-min. intervals for 45 min.	1104	1000	893	819
		Temperature, 20°		
Continuous shaking for 45 min., 1000 cc. of suspension in a 2000 cc. flask.	1124	1014	885	835
Continuous shaking for 45 min., 1000 cc. of suspension in a 1000 cc. flask.	1178	1036	911	848
Flask shaken at 10-min. intervals for 45 min.	1181	1033	929	857
Continuous shaking for 7 hr., 1000 cc. of suspension in a 2000 cc. flask.	1049	955	854	784
Flask shaken at 10-min. intervals for 7 hr.	1071	951	854	772

TABLE X

EFFECT OF METHOD OF EXTRACTING ELECTROLYTES FROM A FLOUR-WATER SUSPENSION ON THE QUANTITY OF NITROGEN EXTRACTED. EXTRACTIONS WERE MADE AT 35°C.*

Treatment	Nitrogen removed							
	Flour-water concentration							
	14	16	18	20	14	16	18	20
	cg.	cg.	cg.	cg.	%	%	%	%
Flask shaken at 10-min. intervals for 45 min.	8.80	9.40	9.55	10.00	29.0	27.7	24.4	23.0
Flask shaken continuously for 45 min., 1000 cc. of suspension in 1000 cc. flask.	8.80	10.05	9.95	10.95	29.0	28.3	25.4	25.2
Flask shaken continuously for 45 min., 1000 cc. of suspension in 2000 cc. flask.	12.05	14.55	14.30	15.20	39.7	41.9	36.6	35.0

*The original nitrogen content of the flour was 2.17 per cent.

TABLE XI

RESISTIVITY OF SOLUTIONS OF KH_2PO_4 AND OF K_2HPO_4 OF DIFFERENT MOLARITY

Molarity	Resistivity of KH_2PO_4 at 28°C.	KH_2PO_4 per liter	Resistivity of K_2HPO_4 at 28°C.	K_2HPO_4 per liter
M x 104	ohms	gm.	ohms	gm.
10.00	838	0.1362	391	0.1743
7.50	1093	.1021	522	.1307
5.00	1580	.0681	788	.0871
4.37	1771	.0595	885	.0762
3.75	2042	.0511	1050	.0654
2.50	2827	.0340	1561	.0436
2.18	3248	.0297	1738	.0381
1.87	3707	.0255	2051	.0327
1.25	5042	.0170	2965	.0218
1.09	5654	.0149	3414	.0190
0.93	6126	.0128	3901	.0163
0.62	7948	.0085	5293	.0109
0.54	8325	.0074	6094	.0095
0.46	9770	.0064	6723	0.0082
0.31	11623	0.0042	....	.....

TABLE XII

VISCOSITIES OF NON-EXTRACTED FLOUR-WATER SUSPENSIONS MAINTAINED AT DIFFERENT TEMPERATURES FOR ONE HOUR

Temperature	Viscosity: Flour-water concentration gm. per 100 cc. 14	16	18	20	Constant *b*	Constant *a*
°C.	°MacM.	°MacM.	°MacM.	°MacM.		
15	10	17	25	36	3.56	−3.08
20	11.5	18	28	39	3.45	−2.90
25	13	21	31	48	3.62	−3.03
30	16	24	35	48	3.09	−2.34
35	18	25	33	44	2.49	−1.59
40	20	26	34	45	2.26	−1.30
50	21	26	34.5	46	2.29	−1.33
60	33	49	73	107	3.29	−2.27
60	48	70	103	176	3.58	−2.44

TABLE XIII

VISCOSITIES OF NON-EXTRACTED FLOUR-WATER SUSPENSIONS MAINTAINED AT DIFFERENT TEMPERATURES FOR ONE HOUR

Temperature	Viscosity: Flour-water concentration gm. per 100 cc. 15	24	Constant *b*
°C.	°MacM.	°MacM.	
15	12.5	55	3.14
20	15.5	70	3.25
25	17	86	3.44
30	21	90	3.09
35	20	86	3.10
40	22	89	2.97
50	24	87	2.24
55	30	132	3.15
60	57	232	2.98

TABLE XIV

EFFECT OF TEMPERATURE ON VOLUME OCCUPIED BY FLOUR-WATER LAYER IN A FLOUR-WATER SUSPENSION

Twenty-five grams of flour made to a volume of 100 cc. was used in this study.*

Temperature	Volume occupied by flour-water layer when suspension is maintained for 1 hr. at constant temperature, then allowed to settle at 1 × gravity for 1 hr.	Volume occupied by flour-water layer when suspension is maintained for 1 hr. at constant temperature, then allowed to settle for 1 hr., then centrifuged at 525 × gravity for 10 min.
°C.	cc.	cc.
20	85.4	32.5
30	80.3	34.9
40	83.1	34.9
50	86.8	39.5
55	88.0	47.8
60	100.0	71.5

*The volume of the flour-water layer determined by centrifuging immediately after preparation was found to be 35.5. Water at room temperature (25°C. app.) was used in this case.

TABLE XV

EFFECT OF FLOUR-WATER RATIO ON VOLUME OCCUPIED BY THE FLOUR-WATER LAYER

The suspensions were maintained at 60° for 1 hour.

Treatment	Volume occupied by centrifugate	Volume to which flour-water layer settled when allowed to stand one hour
	cc.	cc.
20 grams of flour + 70 cc. of water maintained at 60° for 1 hr., then made to 100 cc. and centrifuged immediately.	60.4	
20 grams of flour + 85 cc. of water maintained at 60° C. for 1 hr., then made to 100 cc. and centrifuged immediately.	71.5	
20 grams of flour + 70 cc. of water maintained at 60°C. for 1 hr., then made to 100 cc. and allowed to stand 1 hr. before centrifuging.	65.5	81.4
20 grams of flour + 85 cc. of water maintained at 60°C. for 1 hr., then made to 100 cc. and allowed to stand for 1 hr. before centrifuging.	82.7	87.1

TABLE XVI

VISCOSITY OF NON-EXTRACTED FLOUR-WATER SUSPENSIONS

The flour was kept in contact with different volumes of water at 60° for 1 hour and then the total volume was made to 100 cc. and the viscosity determined.

Treatment	Viscosity	
	Flour concentration	
	20	15
	°MacM.	°MacM.
85 cc. of water + flour made to 100 cc. after 1 hour.	162	
85 cc. of water + flour made to 100 cc. after 1 hour (acidulated).	367	187
70 cc. of water + flour made to 100 cc. after 1 hour.	108	92
70 cc. of water + flour made to 100 cc. after 1 hour (acidulated).	217	162

TABLE XVII

EFFECT OF CHANGING TEMPERATURE DURING TIME OF EXTRACTION OF ELECTROLYTES ON VISCOSITY AND RESISTIVITY OF THE EXTRACT

Twenty grams of flour was used.

Treatment	Viscosity	Resistivity			
		1000 cc. decantate	500 cc. decantate	Decantate after extracting with 1000 cc. + 500 cc. and centrifuging	Second 500 cc. decantate
	°MacM.	ohms	ohms	ohms	ohms
1. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at temperature of 40°C.	257	766.54	4178.32	3151.02	
2. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. + 500 cc. of water for 1 hr. at temperature of 40°.	267	760.26	4429.65	2984.52	14,451.36
3. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at 40° and then suspension maintained at 6°C. for 1 hr.	272	763.40	4084.08	4995.14	
4. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at 40°, then 500 cc. of water at 6°C. for 1 hour.	297	.....	.....	.....	
5. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at a temperature of 20°C.	134	790.11	3958.41	2481.86	
6. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at 20°C. and held at 20°C. for 1 hr.	168	790.11	3958.41	1542.52	
7. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at 20°C. and then the suspension maintained at 40° for 1 hr.	122	794.82	3644.25	904.78	
8. Electrolytes extracted with 1000 cc. of water for 45 min. + 500 cc. of water for 15 min. at 20° and then 1000 cc. of water for 1 hour at 40°C.	310	.....	.....	.....	

TABLE XVIII

EFFECT OF COOLING AND CENTRIFUGING ON RESISTIVITY OF DECANTATE

Method of preparing decantate	Resistivity at 28°C.
	ohms
1. Decantate obtained from a suspension of 20 gm. of flour in 1000 cc. of water maintained at 40°C. for 1 hr., with shaking at 10-min. intervals	757
2. Decantate from 1, boiled for 20 min.	753
3. Decantate from 1, cooled to 5°C. then centrifuged	753
4. Residue from 1, centrifuged, and decantate obtained	694
5. Another residue from 1, cooled to 5°C., centrifuged, and decantate obtained	829
6. Decantate obtained from a suspension of 20 gm. of flour in 1000 cc. of water maintained at 20°C. for 1 hr.	788
7. Decantate from 6, boiled for 20 min.	782
8. Decantate from 6, cooled to 5°C. then centrifuged	782
9. Residue from 6, centrifuged and decantate obtained	703
10. Another residue from 6, cooled to 5°C. then centrifuged, and decantate obtained	753

TABLE XIX

VISCOSITIES OF FLOUR-WATER SUSPENSIONS MAINTAINED AT 20°, OR AT 40°C. FOR 24 HOURS AND THEN THE ELECTROLYTES REMOVED BY EXTRACTING WITH VARYING QUANTITIES OF DISTILLED WATER

Eighteen-gram portions of flour were used.

Total wash water used	Viscosity	
	Suspension maintained at 20°C. for 24 hr.	Suspension maintained at 40°C. for 24 hr.
Liters	°MacM.	°MacM.
1.5	167	156
2	175	172
3	197	178
4	208	176
5	205	186
6	189	180
7	....	179

TABLE XX

VISCOSITIES OF FLOUR-WATER SUSPENSIONS EXTRACTED AT 40°C. FOR 1 HOUR WITH 1000 CC. OF WATER, THEN MAINTAINED AT 6° OR AT 40°C. FOR 24 HOURS, AND FINALLY WASHED WITH VARYING VOLUMES OF DISTILLED WATER

Eighteen-gram portions of flour were used.

Total wash water used	Viscosity	
	Flour-water suspension maintained at 6° for 24 hr.	Flour-water suspension maintained at 40° for 24 hr.
Liters	°MacM.	°MacM.
1.5	213	165
2	233	186
3	228	198
4	225	203
5	210	185
6	202	172
7	197	

ERRATA

In my paper on "The Problem of Test Bakes, with a Discussion of Certain of Their Chemical and Physical Aspects," published in Cereal Chemistry, Vol. 3, on page 209, is found the statement that Jessen-Hansen found the "optimum pH for bread making is about 5, a little higher for choice flours and a little lower for those of lesser quality." This is true if applied to the C_H, but with respect to the pH this should read that the "optimum pH for bread making is about 5, a little lower for choice flours and a little higher for those of lesser qualities."

FREDERICK L. DUNLAP.

THE QUANTITATIVE ESTIMATION OF GLUTENIN IN WHEAT FLOUR.[1]

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In a previous communication Blish and Sandstedt (1925) reported a method for the direct quantitative estimation of glutenin in wheat flour. Later work (1926) has shown that by slightly varying certain details of the specified procedure, especially the ratio of flour to concentration of NaOH used for dispersing the proteins, variable glutenin results are obtained. If the original procedure is strictly observed, the results consistently agree closely with those obtained by the indirect method of Sharp and Gortner (1923) which in the light of our present knowledge of the flour proteins, must be regarded as reasonably accurate. Altho the principles underlying the procedure of Blish and Sandstedt (1925) appear to be sound, it is admittedly fortunate that they happened to hit upon the correct ratio of flour to NaOH concentration, when it is considered that varying this ratio, without otherwise deviating from the principles of the method, will significantly alter the results. In view of these circumstances, together with the fact that the ultimate object of these investigations is the establishing of an "official" method for glutenin in wheat flour, further work has been done in an effort to confirm or disprove the accuracy of the method of Blish and Sandstedt (1925).

This report is presented for two chief purposes. (1) It offers strong circumstantial evidence in support of the accuracy of the glutenin methods of Sharp and Gortner (1923), and of Blish and Sandstedt (1925). (2) It recommends as a method for the quantitative estimation of glutenin in wheat flour an entirely new procedure which combines, to the highest degree, accuracy with ease and simplicity of operation.

Since, as indicated in a report to the Association of Official Agricultural Chemists (1926), slight alterations of apparently trivial details of the Blish and Sandstedt (1925) procedure gave varying glutenin results, it was considered desirable to obtain on a fairly large scale some protein fractions which are produced by varying the method as previously noted, and to subject them indi-

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vidually to analysis by the well known procedure of Van Slyke. Accordingly, several "fractions" were carefully prepared, appropriate amounts were hydrolysed, respectively, and the products of hydrolysis were estimated by Van Slyke's procedure. The results were unsatisfactory and inconclusive. This work merely served to indicate that the Van Slyke method, altho the best available, is not sensitive and accurate enough to detect the extent to which a given protein may be contaminated with small amounts of another protein. By way of further emphasis on this point, Blish (1926) called attention to variations in the results obtained by different workers using the Van Slyke method on different preparations of the same proteins. It seems that there is as yet no method for estimating products of protein hydrolysis, which is of sufficient precision for use in judging the accuracy of procedures for the quantitative separation of individual proteins (excepting within wide limits).

As slight contaminations of one flour protein by another cannot be accurately detected by physical or chemical means now available, one must rely chiefly on Osborne's (1907) characterization of the flour proteins, based upon their solubilities in various solvents. For quantitative purposes, the precautions necessary for the use of these solvents, in order to reduce errors arising from certain "overlapping solubilities," have been stressed in previous reports. These precautions are observed in Sharp and Gortner's (1923) indirect glutenin method, and results secured by this method, or by any method giving similar values must be regarded as the most accurate obtainable for glutenin, in the light of our present knowledge of the identity, individuality, and properties of this protein.

Experimental

In view of the conditions briefly indicated in the foregoing statements and discussion, the only recourse has been to secure additional circumstantial evidence bearing upon the accuracy of the two already existing glutenin methods. In securing this evidence two additional methods of procedure have been devised. Both methods give results which agree closely with those obtained by the Sharp and Gortner (1923), and the Blish and Sandstedt (1925) methods, and yet they radically differ in their technic, both from each other and from either of the two established methods.

Method I

Method I, or the "ammonia method" was calculated to meet the situation in connection with the Blish and Sandstedt (1925)

method, whereby deviations from the specified ratio of flour to NaOH concentration were found to give correspondingly varying glutenin values, due in part, at least, to varying amounts of salt present upon neutralization of the alkali. Salt effect was here eliminated by using ammonium hydroxide as the dispersing medium, the ammonia being subsequently removed by aeration, and the only salts present were those extracted from the flour itself. Eight gm. of flour in a 200-cc. flask was digested (preferably in a shaking machine) at room temperature with 50 cc. of dilute ammonium hydroxide, the proteins being dispersed in this reagent. Ninety-six per cent methyl alcohol was then gradually added with mixing to the 200 cc. mark (plus an additional 5 cc. to allow for the volume of the flour). As soon as the starch had settled, 50 cc. of the supernatant liquid was withdrawn, and introduced into a 100-cc. centrifuge tube. This tube was placed in a water-bath at 50° C., and aerated vigorously with CO_2-free air, for about 2½ hours. During this aeration, methyl alcohol was occasionally added to prevent foaming. The ammonia was entirely removed by this treatment, so that the suspension was neutral to brom thymol blue. When made up to approximately its original strength with methyl alcohol and shaken vigorously, the gliadin which separated out during aeration re-dissolved, and the glutenin settled rapidly to the bottom. The tube was then whirled in the centrifuge, the supernatant liquid poured off, the glutenin transferred to a Kjeldahl flask and subjected to the usual nitrogen determination.

When this procedure was carefully employed, using a standard 95% baker's flour, the glutenin results checked each other, regardless of the concentration of ammonia used for dispersing the glutenin (within the limits tried), and these also checked closely with results obtained by the Blish and Sandstedt (1925) method with the same flour.

Tables I and II show the extent to which glutenin results are affected by varying the ratio of flour to NaOH concentration in the Blish and Sandstedt (1925) method, while with the ammonia method (Table II), the results check with those obtained by Blish and Sandstedt's specified procedure, in addition to being unaffected by varying the ratio of flour to concentration of ammonia used in dispersing glutenin.

Regardless of the ratio of flour to ammonia concentration, all the glutenin percentages in Table II agree closely with the first glutenin figure in Table I, in the securing of which the specified procedure of Blish and Sandstedt (1925) was followed.

TABLE I

BLISH AND SANDSTEDT PROCEDURE

Weight of flour	NaOH concentration*	Glutenin in flour
gm.		%
8	0.10N	4.44†
8	.05N	4.73
8	0.02N	5.01

*Before the addition of methyl alcohol.
†Specified procedure.

TABLE II

AMMONIA METHOD

Weight of flour	NH_4OH concentration*	Glutenin in flour
gm.		%
8	0.2N	4.41
8	.4N	4.36
8	.6N	4.42
8	0.8N	4.44

*Before the addition of methyl alcohol.

Before dismissing the ammonia method, two objections to it should be mentioned. The chief one is that it is a long procedure, requiring very careful manipulation, and therefore objectionable as a routine method. The other is that ammonia does not disperse all of the flour protein, about 15 to 20% being unextracted, and there is no direct proof that all of the glutenin is dispersed. The data in Tables I and II indicate, however, that the glutenin itself is completely dispersed, and that no appreciable portion of it remains in the unextracted residue.

Method II

Method II, or the "barium hydroxide method," is an outgrowth of certain observations made in this laboratory with regard to the solubilities of the barium and calcium "salts" of glutenin and gliadin, respectively, in methyl alcohol. When three volumes of strong methyl alcohol are added to one volume of a solution of glutenin in $Ba(OH)_2$, the glutenin is completely precipitated. When a solution of gliadin in $Ba(OH)_2$ is similarly treated, the gliadin does not immediately precipitate, but, upon standing, it does so very slowly and incompletely. $Ca(OH)_2$ solutions of glutenin and gliadin, respectively, behave in a similar manner. When this principle was applied to flour, as will be described later, the use of $Ba(OH)_2$ gave glutenin results which were in very good concordance with results obtained by any of the three methods already discussed. When $Ca(OH)_2$ was used, appreciably lower results were generally obtained, for some unknown reason, altho they occasionally approximated very closely the glutenin values secured by the use of the other methods.

Recommended Procedure

Weigh 8 grams of flour into a 200-cc. flask. Add with spatula 0.2 gram powdered $Ba(OH)_2$ .8 H_2O, followed by 50 cc. water. Digest for 1 hour with frequent shaking. Then add 96% methyl alcohol, so that when the material is thoroly mixed there will be 5 cc. of liquid above the 200 mark (to correct for the volume of the flour). The starch immediately settles to the bottom, after which pour supernatant liquid *at once* through a cotton plug, and immediately withdraw 50 cc. for Kjeldahl nitrogen determination. Convert the nitrogen to protein by the factor 5.7, subtract the percentage of protein in the extract from the percentage of total protein in the flour, and the result is the percentage of glutenin in the flour. It is to be observed that the 50-cc. aliquot portion of the filtrate must be taken immediately, because after 10 or 15 minutes the filtrate will begin to be cloudy, owing to a very slow precipitation of the gliadin.

The use of this method, as compared with the Blish and Sandstedt (1925) method, with a number of flour samples, showed in almost every case a striking concordance of results. This is shown in Table III.

TABLE III

COMPARISON OF BARIUM HYDROXIDE METHOD WITH BLISH AND SANDSTEDT METHOD

Flour No.	Total protein	Glutenin by $Ba(OH)_2$ method	Glutenin by Blish and Sandstedt method
	%	%	%
940	11.00	4.64	4.67
961	10.66	4.56	4.46
963	11.69	5.10	5.06
970	12.31	5.59	5.47
973	11.46	5.14	5.11
981	9.69	4.76	4.73
920	11.23	4.28	4.30
922	10.92	4.10	4.20
980	8.66	3.50	3.39
999	11.51	4.39	4.44

In preliminary determinations, with flours of average protein content, it appeared that good results were secured regardless of the amount of $Ba(OH)_2$ used, and the dry, powdered reagent was merely added with a spatula, without paying any special attention to the amount. Dr. D. A. Coleman,[2] of the Grain Research Laboratory of the Bureau of Agricultural Economics, U. S. Department of Agriculture, after trying the method, reported that in the case of some very high protein flours, glutenin values varied inversely with the amount of $Ba(OH)_2$ used, but he obtained very

2 Private communication.

satisfactory results with flours of low protein content, without regulating the amount of $Ba(OH)_2$. This point was therefore investigated in this laboratory. Dr. Coleman's observation that, especially in high protein flours, the glutenin decrease as the amount of $Ba(OH)_2$ is increased, was confirmed. When 0.2 gm. $Ba(OH)_2 .8H_2O$ were used, satisfactory results were obtained. 0.6 gm. gave decidedly lower values. 0.1 gm. appeared to be inadequate for satisfactory dispersion of the proteins. 0.2 gm. portions are therefore recommended, and this is the amount used in obtaining the data in Tables III and IV, respectively.

Table IV, which follows, offers a comparison of glutenin values obtained with several flours of high protein content, by the method of Sharp and Gortner (1923), the method of Blish and Sandstedt (1925), and the $Ba(OH)_2$ procedure.

TABLE IV

COMPARISON OF METHODS WITH FLOURS OF HIGH PROTEIN CONTENT

Flour No.	Total protein	Glutenin by Blish and Sandstedt method	Glutenin by barium hydroxide method	Glutenin by Sharp and Gortner method
	%	%	%	%
587	14.65	5.73	5.97	6.02
990	15.44	5.75	6.46	6.26
995	14.08	5.70	5.88	6.22
996	14.14	5.70	6.01	6.11
997	14.31	5.75	5.82	5.99
1010	15.61	6.30	6.38	6.61
574	16.89	7.21	7.06	....

Data in Table IV indicate, for the most part, a very good agreement between results obtained by the $Ba(OH)_2$ and the Sharp and Gortner (1923) methods, respectively, for flours of unusually high protein content. The Blish and Sandstedt (1925) procedure tends toward slightly lower results, in most cases, with these flours, altho in several instances the agreement among all three methods is excellent.

When it is considered that in this work one is dealing with quantitative protein separations, and that all values are based upon estimations of total nitrogen (multiplied by the factor 5.7) in each fraction, respectively, the agreement among values obtained by these different methods may be regarded as fairly satisfactory. This concordance of results secured by the different methods is sufficiently close to furnish very strong circumstantial evidence as to the reasonable accuracy of any one of them. The "barium hydroxide" method easily takes precedence over the others from the

standpoint of ease and simplicity of operation, to say nothing of economy of time and apparatus.

There is, of course, a question as to what happens to the albumin and globulin in the barium hydroxide method. Altho positive proof is lacking, there is evidence that these proteins are largely in the filtrate, for the protein material therein, after hydrolysis, yielded 24.6% of its total nitrogen in the form of ammonia nitrogen, in the case of a representative sample of flour. This figure is lower than the accepted value for pure gliadin to approximately the extent which would be expected providing a small amount of albumin and globulin were present; for pure gliadin, after hydrolysis, yields 26% of its nitrogen as ammonia nitrogen, while the ammonia nitrogen figures for albumin and globulin, respectively, are 6.8 and 7.7. Therefore, in a mixture of these three proteins, the percentage of ammonia nitrogen, after hydrolysis of the mixture, may be expected to reflect the extent to which albumin and globulin were present in the original mixture. Incomplete precipitation of glutenin would also cause a lowering of the ammonia figure, since the amide nitrogen content of glutenin is approximately 15%, in terms of the total glutenin nitrogen. However, experiments with purified glutenin indicated that precipitation is complete under the prescribed conditions.

Summary

1. A new and exceedingly simple method for the quantitative estimation of glutenin in wheat flour is presented and rcommended and is herein referred to as the "barium hydroxide method."

2. The new method is superior to other procedures from the standpoints of simplicity and economy of time, as well as equipment.

3. That the methods of Sharp and Gortner (1923), and Blish and Sandstedt (1925), respectively, are reasonably accurate is strongly evidenced by experiments herein reported.

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A STUDY OF DURUM WHEATS[1]

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Durum wheats have long been cultivated in southeastern Europe and northern Africa, but their introduction into the United States dates from the closing years of the last century. The earlier American investigators were seemingly desirous of establishing the durums as bread-making wheats. Carleton (1901), and Shepard (1903, 1905, 1906), emphasized the desirable properties of flours milled from durum wheat, which adapt them to the production of yeast-leavened bread. Other enthusiasts, including Christadoro, endeavored to interest commercial bakers in these flours.

Despite these efforts, durum wheat flour was found unsuited for the ordinary bread of commerce unless mixed with a large percentage of vulgare, or common, wheat flour. Bread made from durum flour was generally denser in texture, darker in crumb color, and in general failed to conform to the usually accepted standards of superior bread. Durum wheat products are used largely in the manufacture of macaroni, spaghetti, and other edible pastes, however, and a considerable tonnage of durum semolina is converted annually into such products in America.

In view of the lack of exact information respecting the differences in the biochemical characteristics of vulgare and durum wheats which cause the observed differences in baking properties, a study of the flours milled from durum wheat was undertaken. Such flours have not been so extensively studied as the vulgare wheat flours, particularly by means of certain new methods and equipment.

Proteins of Durum Wheat Flours

Durum wheat marketed in the United States contains the highest average percentage of protein of any of the commercial classes of wheat. This is evident from the data of Thomas (1917), and Schollenberger and Clark (1924). It is probable that this is largely due to the effect of the climate of the area in which durum is chiefly grown. Ladd and Bailey (1910) found no appreciable difference in the pro-

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tein content of durum and of bluestem (common or vulgare) wheat grown on the same farms, and for a period of four seasons. In a single crop season (1911) Ladd (1912) observed that the average protein content of durum wheat grown on five farms in North Dakota was somewhat less than one per cent higher than that of common spring wheat grown on the same farms.

All but two of the durum wheat flours used in our experiments (Nos. 10 and 12) were high in protein content. Half of the samples contained 13% or more of crude protein on the moisture-free basis (including No. 17, which is reported as containing 12.97%), and one-third of the number contained more than 14%.

The percentage of glutenin was determined in eighteen durum flours from several sources, using the method of Blish and Sandstedt (1925). The data are recorded in Table I. Glutenin content was high in the flours which contained a large percentage of total protein. Ratio of glutenin to protein was then computed, with the results recorded in the same table. With the exception of three samples (Nos. 2, 5, and 18) this ratio varied within the limits of 0.34 and 0.40. Two-thirds of the samples ranged within the relatively narrow limits of 0.36 and 0.40. The differences in this respect are so small, in view of the relatively large experimental error of the glutenin method, as to indicate that the glutenin-protein ratio will hardly serve to classify durum flours on the basis of their adaptability to bread making or macaroni production.

The glutenin-protein ratio is not sufficiently different from that of vulgare wheat flours to serve to distinguish between the two classes of flour. A parallel study with vulgare wheat flours resulted in glutenin-protein ratios ranging between 0.36 and 0.39, and averaging 0.378, while the average for the eighteen durum flours examined was 0.365. This difference is practically within the range of experimental error of the glutenin determination and, in any event, is not great enough to account for the relatively large difference in the type of bread produced from the two classes of flour.

Viscosity of Acidulated Durum Flour Suspensions, and Glutenin Quality Constant *b*

Sharp and Gortner (1923) advanced the opinion that the viscosity of acidulated suspensions of flour in water may be used to measure the "quality" of glutenin. Several ratios of flour to water are used in such determinations, the procedure being detailed by Gortner (1924). The quality constant *b* is the angle with the hor-

TABLE I

COMPOSITION OF DURUM WHEAT FLOURS AND RESULTS OF VISCOSITY AND EXTENSIMETER DETERMINATIONS

Sample No.	Ash	Crude protein (Nx5.7)	Glutenin	Glutenin / Protein	Constants "b"		Viscosity of 21 grams of flour per 100 cc.		(c) / (d)	Extensibility of dough
					Extracted suspensions	Unextracted suspensions	Extracted suspensions (c)	Unextracted suspensions (d)		
	%	%	%				°MacM	°MacM		
1	0.83	14.89	5.72	0.385	2.36	1.82	114	22	5.18	12.76
2	.72	14.95	4.88	.327	3.55	2.78	59	21	2.81	.....
3	.74	14.17	5.23	.369	3.15	3.13	51	13	3.98	.....
4	.60	14.31	5.50	.394	3.16	3.02	51	31	1.64	.....
5	.50	11.87	3.72	.313	3.70	3.48	55	15	3.66	13.50
6	.64	12.58	4.57	.361	3.32	2.41	74	25	2.96	9.23
7	.82	14.94	5.32	.356	3.05	2.60	69	24	2.88	10.70
8	.81	12.76	4.66	.367	3.36	2.68	76	32	2.37	9.09
9	.75	14.59	5.87	.402	2.78	2.32	86	29	2.99	10.90
10	.74	10.94	4.10	.374	2.12	2.68	42	17	2.47	15.60
11	.64	16.59	5.62	.339	3.00	2.09	97	48	2.02	11.20
12	.69	10.51	3.95	.375	2.90	3.28	49	19	2.57	12.20
13	.69	12.40	4.70	.380	2.48	2.79	63	21	3.00	10.83
14	.70	12.41	4.79	.383	3.05	3.43	118	39	3.02	9.80
15	.72	12.22	4.85	.397	3.21	3.79	104	32	3.25	10.20
16	.63	12.69	4.71	.371	2.93	2.92	57	20	2.85	10.52
17	.80	12.97	4.99	.385	2.61	3.16	84	24	3.50	10.51
18	0.86	13.16	3.82	0.290	2.23	2.47	74	16	4.63	10.96
Av.	0.72	13.27	4.83	0.365	2.87	2.79	73	24	3.09	11.20

izontal formed by the integrated curve or straight line plotted, with logarithm of viscosity as ordinates, and logarithm of concentration as abscissas. This represents the increase of logarithm of viscosity per unit of increase of the logarithm of flour concentration, in grams.

This constant *b* bears no definite relation to the actual viscosities of the several preparations. Thus, a flour might yield preparations with relatively low viscosities and yet have a relatively high quality constant *b*. This possibility was emphasized by Gortner (1924), who demonstrated its occurrence in one of the two flours which he compared. As a measure of the actual quality of the glutenin, the constant *b* was deemed by him to be of the greatest value.

TABLE II

CHANGE OF VISCOSITY IN DEGREES MACMICHAEL WHEN A 21-GRAM PORTION OF FLOUR 1 WAS WASHED WITH VARYING QUANTITIES OF DISTILLED WATER

Liters of water	0	1	2	3	4	5	6	7	8	9	10
Degrees MacMichael Determination I	22	89	139	147	174	180	172	197	216	204	213
Degrees MacMichael, Determination II	22	97	132	149	163	168	176	195	209	202	187

Numerous flour chemists utilize the actual viscosity of acidulated flour suspensions as an index of baking quality. The flours are sometimes leached with water before acidulation. The majority of analysts do not leach their flours before determining viscosity. however. That substantial increases in viscosity result from the preliminary leaching with water was demonstrated by Sharp and Gortner (1923), who urged that this treatment be accorded the preparations in order to eliminate one variable, namely, the salts and other extractives of the flour. It is contended that the effect of leaching upon viscosity results from the removal of salts by the water.

The influence of the removal of electrolytes from the durum flour was studied by washing or leaching 21-gram portions of Flour No. 1 with various quantities of distilled water. The determination was made by varying the leaching treatment by increments of one liter from 0 to 10 liters. With the first liter the time of extraction was one hour, and with all subsequent extractions, 15 minutes. The wash water was decanted in each instance after the flour particles had settled.

Viscosity of the acidulated flour suspension after each extraction is given in Table II. The viscosity increased up to eight ex-

tractions, after which it decreased. This tends to confirm the observation of Sharp and Gortner (1923) who (p. 76) did not follow exactly the same procedure but, using 500-cc. portions of water, encountered increases in viscosity up to twelve extractions.

These observations lend emphasis to the importance of flour electrolytes in determining viscosity. With this 50% increase in viscosity between the second and eighth extraction, it might be deduced that variations in the quantity and nature of the flour salts which depress viscosity would complicate the method and make a duplication of results difficult.

Recognizing this difficulty, an effort was made to follow a uniform procedure in the treatment of all samples subjected to the viscosity determination. The flour suspensions were stirred at a uniform rate and for a definite time while adding the lactic acid. Triplicate readings were then taken, the suspensions being stirred between readings. The reading, in degrees MacMichael, with the viscometer cup rotating at constant speed and after the bob had ceased to swing past the "mean," was noted in each of the triplicate readings, and the largest of the three values was recorded. In other respects the procedure detailed by Gortner (1924) was followed.

It should be noted, however, that larger concentrations of durum flour were required than of vulgare wheat flours, because of the relatively lower viscosity of suspensions of the former. It was necessary at times to use as much as 30 grams of flour per 100 cc. of suspension in order to have a sufficient range of concentration to compute the constant b.

In addition to the determination of the leached or extracted flour suspensions, parallel measurements were made with unextracted suspensions. Constant b of the latter was computed, and the resulting data are recorded in Table I. The difference between the averages of the two series of constant b is not great, the numerical value being slightly higher in the extracted suspensions. These data indicate that there is no correlation between the two series of observations. A similar lack of correlation was previously noted by Johnson and Bailey (1924).

The magnitude of the constants b of these durum flours is within the range previously reported for vulgare wheat flours. Sharp and Gortner (1923) reported on eleven flours which ranged from $b = 2.206$ to $b = 3.048$, with an average of $b = 2.633$. Johnson and Bailey examined fourteen soft winter wheat, or cracker, flours

which ranged from $b = 2.57$ to $b = 4.00$, and averaging $b = 3.36$. Grewe (1925) collected 16 samples of vulgare wheat flour from various regions in the United States. A range of $b = 2.16$ to $b = 2.90$ was observed, with an average of $b = 2.49$. All these values were obtained after leaching, or extracting, the flours to remove electrolytes. The average constant b for durum flours similarly treated was 2.87, which is intermediate between the extremes of the averages reported by the other observers for vulgare wheat flour. Accordingly, it appears that the durum flours are not sufficiently distinctive in this particular to make it possible to utilize the constant b as a means to differentiate the two types of wheat. Nor is the constant b correlated with baking strength (as recorded in terms of loaf volume) so far as can be determined from the baking tests of seven of the durum flours reported in Table III.

TABLE III

RESULTS OF BAKING TESTS OF 75% EXTRACTION DURUM PATENT FLOURS MILLED FROM 1924 CROP WHEATS

Sample No.	Variety	Grown on Exp. Station farm at	Loaf volume	Expansimeter test	Absorption	Color	Texture
			cc.	cc.	%	Score*	Score
9	*Kubanka	Crookston, Minn.	1900	750	56.9	96g	98
7	Mindum No. 470	Waseca, Minn.	1900	800	56.9	97y	99
11	Mindum	Morris, Minn.	1880	700	56.0	97y	98
8	Mindum No. 470	Grand Rapids, Minn.	1830	600	56.4	94y	92
12	Mindum No. 470	University Farm, Minn.	1910	770	53.6	97y	97
10	Pentad No. 1968	University Farm, Minn.	1720	930	54.7	95	97
6	Mindum	Crookston, Minn.	1840	600	55.3	95y	95

*g = gray, y = yellow.

Many flour chemists determine the viscosity of a single preparation or flour suspension. In some laboratories the practice is to extract or leach the flour with water, while in others no preliminary leaching is accorded the flour, which is merely suspended in water and then acidulated. For this reason it was deemed advisable to record the viscosities of certain durum flour suspensions, and the suspension of 21 grams of flour made to 100 cc. was selected. The viscosities, in degrees MacMichael, as determined under a uniform set of conditions are recorded in Table I. The ratio between the viscosity of the extracted and the unextracted flours was computed in each instance and the resulting data are also recorded in the table.

No correlation can be discerned between the protein content (Table I) and the viscosity of the extracted flour suspensions. Flour No. 11, which contained 16.59% of protein, when leached and acidulated gave a suspension that was no more viscous than that

produced from certain flours which contained less than 12.5% of protein (Nos. 14 and 15). Again, in flours 13 and 14, which contained practically the same percentage of protein and also of glutenin, the viscosity of the extracted suspensions varied nearly 100%. A lack of correlation between the unextracted suspensions and protein content will also be observed.

Reference has already been made to the relatively large increase in viscosity effected by leaching durum flour. The two extractions in the determinations recorded under column "c" in Table I effected an increase in viscosity which ranged around 300%. The increase was not uniform, however, and the ratio of the viscosity of "c", or extracted, to "d", or unextracted, appeared to vary with the ash content. The coefficient of correlation of the ratio $\frac{c}{d}$ to percentage of ash was found to be 0.462 ± 0.0125. This is a significant positive correlation, yet it is hardly great enough to justify the substitution of the ratio $\frac{c}{d}$ for the ash determination as an index of the degree of refinement of the flour.

It has been shown that durum wheat flour commonly contains a higher percentage of ash than vulgare wheat flour. Ladd and Bailey (1911) reported an average of 0.47% of ash in patent flour milled from hard red spring (vulgare) wheat, and 0.68% in patent flour of about the same extraction percentage milled from durum wheats. The average ash content of a series of durum wheat flours examined by Shollenberger and Clark (1924) was 0.77%, which is the highest ash content of flours from any of the market classes of wheat.

In the series of durum wheat flours used in these experiments the average ash content was 0.72%. It is quite probable that this high content of ash is largely responsible for the low viscosity of unextracted durum flour suspensions after acidulation. Two extractions may fail to remove all the inorganic electrolytes of durum wheat flour which may tend to depress the viscosity of the suspensions so extracted and subsequently acidulated. The average concentration of durum flour in the suspension must be increased above that which is required when vulgare flours are used in the determination of constant *b*. The low viscosity of leached and acidulated suspensions is the most characteristic property of durum wheat flours, aside from color, which serves to distinguish them from vulgare wheat flours of the same percentage extraction.

Extensibility of Durum Wheat Flours

Chopin (1921) described an instrument designed to measure the extension of dough which can be effected when the plane surface is stretched to cover the surface of a hemispherical body. Bailey and Le Vesconte (1924) published a translation of Chopin's paper, together with the results of their experience in using the extensimeter. The extensibility of three hard winter wheat flours which they tested ranged between 13.8 and 14.9; while with spring wheat flours values to 18.5 were recorded.

Fifteen of the eighteen samples of durum flour to which reference has been made, were subjected to tests with the Chopin extensimeter. A preliminary study was conducted of the effect of varying the proportion of water in the dough. In general, it was observed that varying the amount of water used in mixing the dough through the working range,[2] varied the extensibility less than 5%. A similar variation in the water in vulgare wheat flour doughs varied the extensibility by 50 to 100%.

The extensibility of fifteen durum wheat flour doughs was substantially less than that of the vulgare wheat doughs previously tested. These values are recorded in Table I, and range from 9.09 to 15.60. All except two of the samples varied within the comparatively narrow limits of 9.1 and 12.8, and the average of the fifteen samples was 11.2. This property of durum wheat flour was accordingly quite distinctive and seems to justify conclusions reached from baking tests and the experience of macaroni manufacturers—that durum flours possess peculiar physical characteristics.

Enough was available of seven of the durum flours for use in experimental baking tests. The results of these tests are recorded in Table III. The coefficient of correlation of extensibility and loaf volume, 0.275 ± 0.006 is much less than that of extensibility with the expansimeter test (as developed by Bailey, 1916) which was 0.928 ± 0.003.

Color of Durum Semolinas and Flour

The color, or visual appearance, of durum semolinas is probably the resultant of at least five variables. These are (1) content of carotinoid pigments, (2) relative density, or vitreousness, of

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2 By "working range" is meant the maximum and minimum proportion of water that can be used in the preparation of what may be regarded as a "dough." With durum flours the range is about 15% of the weight of the flour, while with vulgare flours, it may be as high as 20%.

the particles, (3) size of the particles, (4) pigments contributed by the outer fibrous covering, or bran, of the kernel, and (5) dirt or foreign material. A sixth factor becomes operative when the semolinas are wetted, namely, the production of dark substances or pigments in consequence of oxidation accelerated by enzymes known as oxidases.

In general, the manufacturers of macaroni and spaghetti desire a product which is vitreous, or dense, in texture; and of a faint yellow or creamy hue, but free from gray or brown tints; and free from foreign particles. An instrument capable of quantitatively measuring these properties would be of great service to the industry.

While these experiments were in progress, a K. & E. color analyser, or spectrophotometer, was acquired by the Division. This instrument was so designed as to make it possible to measure the characteristics of light reflected from opaque surfaces as well as light transmitted through transparent bodies. It has been described by Keuffel (1925).

An attempt was first made to measure the properties of light reflected from the surface of a layer of dry durum flour. Eight samples of flour were provided by the laboratory of one of the large durum wheat mills. The flours had previously been scored by the mill laboratory on the basis of the "slick" or Pekar test. Scores of 99 were assigned to the flours of the deepest yellow hue, with progressively lower scores as the yellowness diminished and the flour became duller and grayish in hue.

The percentages of light reflected from the dry flour surfaces in terms of the reference beam reflected from a freshly scraped magnesium carbonate block are recorded in Table IV. In general, the group of flours scoring 96 and 97 reflected more blue light (wave length 480 μμ than the flours scoring 98 and 99. While the values in terms of light reflected do not decrease regularly with increasing color score, the decrease is so close to regular as to justify the conclusion that the relative depth of yellow hue may be determined quantitatively in this manner. Unfortunately, the visibility of light to the human eye is relatively low when the wave length is less than 480 μμ. The Mazda lamp bulbs used with the spectrophotometer are not rich enough in the short wave lengths to allow observation at 460 μμ or less. From the characteristics of the curves which graphically record these data, and from other observations with yellow pigments, it appears probable that the

differences between the several flours would become even more apparent at 460 μμ and 440 μμ. The mercury vapor arc light might be more suitable than the Mazda lamps, as it affords a bright line at 435.8 μμ (see Schertz, 1923).

In the range of wave lengths above 500 μμ no correlation was detected between the color score and reflection of light in this limited series of observations. It is possible that certain properties of durum flours involved in visual appearances might be revealed by a more extended study in this range of the visible spectrum.

One factor contributing to the relative depth of yellow hue in durum wheat flour is the concentration of carotinoid pigments. Clark (1924) determined the gasoline color value, using Winton's (1911) method, and arbitrarily limited durum products suitable for macaroni production to samples with a minimum color volue of 1.30. Schertz (1923) demonstrated the advantage of the spectrophotometer over the colorimeter in estimating the concentration of pigments, hence it appeared advisable to apply the spectrophotometric method to these samples of durum flour.

TABLE IV

WAVE LENGTH, PERCENTAGE OF REFLECTION, AND COLOR SCORES OF EIGHT DURUM FLOURS EXAMINED BY REFLECTED LIGHT FROM DRY SAMPLE

Color score	96	97−	97+	97+	98−	98	99	99
Wave length μμ	%	%	%	%	%	%	%	%
480	72.5	71.7	74.0	71.0	66.2	69.7	68.0	66.0
500	74.5	75.2	79.0	76.9	72.7	75.4	73.6	73.0
520	82.3	82.1	83.1	85.8	82.0	82.7	81.3	82.1
540	81.0	83.8	85.8	85.3	81.9	85.3	83.9	83.6
560	85.9	86.0	88.6	88.7	86.4	85.5	85.1	86.9
580	86.6	86.6	89.4	88.3	86.6	85.8	86.0	87.3
600	86.3	86.3	89.2	89.3	86.3	87.2	87.1	87.9
620	85.4	88.9	89.9	90.3	87.1	87.2	87.4	87.7
640	86.3	87.9	89.4	89.5	87.8	87.2	88.2	88.7
660	89.2	89.7	90.9	90.6	88.7	89.2	88.2	89.0

In these studies, 20 grams of flour was suspended in 100 cc. of clear, colorless high-test gasoline. This was shaken vigorously for a time, and then allowed to stand for 16 hours in the dark. The gasoline extract was carefully pipetted from the containers, and transferred to a 10-cm. tube. This was placed in the tube support of the spectrophotometer, together with a 10-cm. tube containing gasoline alone, and the percentage transmittancy was determined.

The resulting data are recorded in Table V, and are presented graphically in Figure 1. Durum flours which were distinctly yellow and received the highest numerical scores yielded extracts with

gasoline which transmitted less light than the flours which were scored lowest. There was less difference between the flour scored 96 and that scored 97+, than between the flour scored 98— and those scored 99. It is probable that factors of color other than those involved in the carotin content occasion the low score of the flour marked 96.

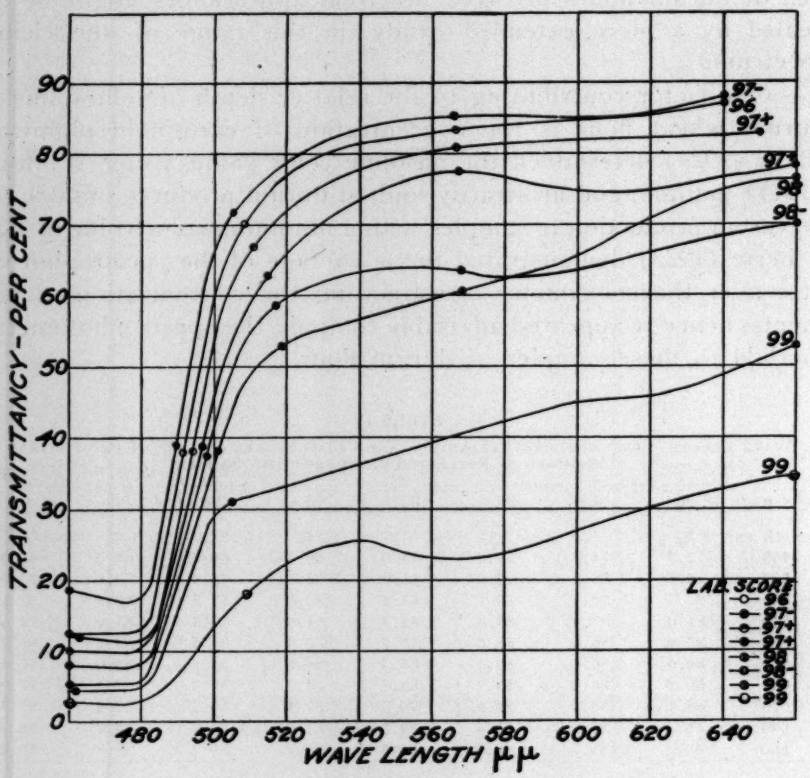

Fig. 1. Transmittancy of Light of Different Wave Lengths Through Gasoline Extracts of Durum Wheat Flours to Which Varying Color Scores Were Assigned.

In general, the correlation between the laboratory score and the data resulting from spectrophotometric analysis of the gasoline extract is fairly definite. The readings in the range of 520 μμ distinguished between the flours to greatest advantage. It is possible that a less concentrated solution of the flour pigments in gasoline would yield spectrophotometer readings which differ to a greater degree than the concentration involved in these studies.

TABLE V

WAVE LENGTH, PERCENTAGE OF TRANSMITTANCY, AND COLOR SCORES OF EIGHT SAMPLES OF DURUM PATENT FLOUR

Color score	96	97−	97+	97+	98−	98	99	99
Wave length $\mu\mu$	%	%	%	%	%	%	%	%
460	12.4	18.7	10.0	12.2	8.2	5.3	2.8	4.3
480	12.7	17.4	10.0	11.1	8.2	5.9	2.8	4.5
490	28.3	35.4	25.0	23.9	21.9	18.3	6.9	14.4
500	60.2	68.2	54.0	48.3	41.7	37.8	13.6	29.9
510	72.5	71.0	67.4	62.7	54.8	49.6	20.5	31.9
520	77.4	76.2	72.4	69.7	60.0	53.0	22.8	33.3
540	81.8	78.8	74.7	73.3	56.8	57.1	25.7	36.0
560	81.4	84.4	79.6	77.7	66.2	59.0	21.1	40.2
580	84.8	84.6	79.1	69.7	61.3	62.7	25.0	41.0
600	84.6	85.2	79.1	75.4	62.6	64.1	29.0	45.7
620	83.7	85.8	.....	75.1	67.6	71.0	31.7	43.8
650	88.2	89.8	83.0	76.2	68.5	75.5	34.0	51.7

Extensibility of durum patent flour doughs, as determined with the Chopin extensimeter, averaged appreciably lower than that of vulgare patent flour doughs.

Light reflected from the surface of compacted durum flour was analyzed with a spectrophotometer and it was found that, in general, the flours to which the highest scores had been assigned ab-

Summary

Durum wheat flour contains a higher average percentage of protein than flours milled from average vulgare, or common, wheats. This is probably the result of the climatic conditions which prevail in the regions in which the durum wheat of commerce is chiefly grown.

Glutenin constituted about the same proportion of the total protein of durum flour as has been reported for vulgare wheat flour. Moreover, there was no substantial variation in the glutenin-protein ratio in the durum flours that were analyzed. This ratio averaged 0.37.

Quality constant *b* of extracted suspensions of durum wheat flours averaged 2.87, which is within the range previously reported for vulgare wheat flours. There was no correlation between constant *b* and protein content of the durum wheat flours.

Viscosity of leached and acidulated suspensions of durum wheat flour was much lower than that of like preparations of vulgare wheat flour of the same concentration. This served to distinguish between the two types of flour more definitely than the constant *b*, or any chemical property or constitutent determined in these studies.

sorbed more of the short wave lengths of light. This is equivalent to a measure of the relative yellowness of the flour.

Concentration of carotinoid pigments was greatest in the durum wheat flours receiving the highest laboratory color scores as evidenced by the low percentage of transmission of light through gasoline extracts of such flours. It appears probable that the analysis of light (1) reflected from the flour surface, and (2) transmitted through gasoline extracts, may serve as an accurate quantitative measure of color properties of such durum products as are of interest to the macaroni industry.

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A RATIONAL BASIS FOR THE STANDARDIZATION OF THE EXPERIMENTAL BAKING TEST[1]

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The writer has the honor to be chairman of the committee on standardization of the experimental baking test of the American Association of Cereal Chemists, and referee on the experimental baking test for the Association of Official Agricultural Chemists. In this dual capacity he has devoted time and study to an analysis of the somewhat conflicting views as to the rational basis of the experimental baking test. As a final outcome, he is forced to a conclusion which he deems of sufficient import and sufficient interest to be submitted for adjudication, even tho the submission may involve, to a small extent, the reiteration of premises stated on previous occasions.

What is, or should be, the function of any so-called "standard test" of a "raw" or partially refined material, from the standpoint of ascertaining its usefulness as the chief constituent of a manufactured product? There is agreement that the function of such a test should be to indicate the extent to which the material in question meets or fails to meet certain definite requirements or specifications and, obviously, these requirements or specifications themselves must be known and understood by the individual who interprets the results of the test.

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It should not be necessary actually to make a specimen of the finished product in order that the purposes of such a test may be served. In fact, this is undesirable, for there are good reasons why such procedure is unsound, misleading, and unscientific, when speaking from a strictly standardization viewpoint. The argument against such a method of procedure lies chiefly in the fact that the manufacture of the finished article may require special skill, experience, or artistic ability, or a combination of these qualities No two individuals possess any of these qualities or any combination of them to the same degree. Such a method of procedure cannot be expected to yield uniform results in any process which involves personal manipulation or judgment.

When the chemist tests iron or steel which is intended for use in making automobile parts, he does not attempt actually to make a few drive shafts or connecting rods, but he determines such properties of the metal as hardness and tensile strength. Investigating a lubricating oil, he does not put some of it in a certain type of automobile, drive the car until a bearing burns out, and then evaluate the oil on the basis of the time and average rate of speed required. He merely determines the oil's viscosity, acidity, resistance to heat, and other properties and is then able to classify the oil with reference to its general utility. It should be emphasized that all details of every standard method are definitely prescribed and must be rigidly followed. A given test is carried out in precisely the same way for every sample of the material under consideration. A specified test, performed on a given sample of material, should always present the same picture, whether repeated by one or by different individuals. The most important consideration is that the only *variable* is the material which is to be tested.

These remarks refer to facts which are so obvious and well known in this day of scientific testing of materials that reference to them may be regarded by some as unnecessary and almost insulting to the intelligence of the average chemist. Nevertheless, it is apparent that the present lack of standardization of our most important flour test is in a very large measure due to failure to appreciate and to apply these elementary principles which are and must always be the very foundation of all standard methods for testing materials. Success in any attempt to devise a standard experimental baking procedure will depend first and last upon the extent to which the foregoing principles can be applied.

The lack of uniformity or anything approaching it, in methods of conducting experimental baking tests is well known. The mere fact that methods differ from each other furnishes no ground for condemning any one of them from the standpoint of its possible utilization as a standard procedure, or from the standpoint of its usefulness to the operator. In considering any method from the viewpoint of its suitability as a *standard procedure* there is, first of all, one requirement to be met, and at present all of our consideration should be and must be concentrated upon this one issue.

If this obstacle cannot be surmounted at the outset, the project cannot succeed. This requirement is that the method shall, within satisfactory limits of error, involve but *one variable* which in this instance is the flour itself. From this fundamental viewpoint, it will be a purpose of this discussion to show wherein the majority of our experimental baking procedures fail to meet this requirement, confining this phase of the argument largely to *types* of procedure rather than to actual methods. Attention will then be directed to the one type of procedure which can logically be considered from the standpoint of meeting this demand.

As stated above, the type of experimental baking method employed by most workers does not meet the essential condition whereby the flour is the only variable in the procedure. In this type of method an attempt is made to bake a loaf of bread having certain definite characteristics, thus combining the test of the material with the manufacture of the finished article. Some technicians strive to produce a "commercial" style of loaf, others seek maximum expansion as an indication of "strength"; some attempt to produce a loaf which will suit the gastronomical inclinations of the boss of the organization, while some aim to bring about a happy combination of these or other qualities.

In any event, definite characteristics are sought, and in order to produce these as nearly as possible, various details of the method are altered in accordance with certain predetermined properties of the flour. Among such predetermined properties, which may be regarded as "diagnostic symptoms," may be mentioned acidity, pH, buffer value, viscosity, crude gluten, absorption, protein, ash, nature of the bleaching process used, previous experience with wheats of particular crops or localities, and time required from the setting of the dough to its falling when "punched."

In addition to the differences of opinion among technicians with regard to the actual and relative values and uses of these various

symptoms, there is no general agreement as to the precise manner in which the dough should be mixed, punched, and molded. In any method of this type, diagnostic and manipulative skill will largely influence the character of the loaf produced. Such a method, then, is clearly a test not only of the flour itself, but also of the technician's ability, or dexterity, or artistry, or diagnostic skill, or any possible combination of these qualities to the same degree. Thus this type involves more than one variable, and thereby fails to meet one of the most essential conditions of all standard tests, which is that there shall be but *one* variable, namely the material itself.

There is no intent to belittle or to the slightest degree deny the value of methods of the foregoing type. Experts and authorities agree and insist that almost any flour can be satisfactorily used in the production of commercial bread, providing it is properly handled. Some technicians are so expert in adjudicating and handling flours that they can generally produce a so-called "commercial" type of loaf from almost any flour by the use of special diagnostic and manipulative skill. This ability is highly commendable, and is to be envied by those of us who, for various reasons, may not have acquired it.

To possess this ability is a decided asset to any cereal chemist, and he will find ample opportunity to exercise his talent, especially for demonstration purposes, for "trouble-shooting," and for use in confirming judgment based upon preliminary tests, or diagnosis. Nevertheless it must be granted that any standard test, so far as its actual performance is concerned, should not demand exceptional manipulative or diagnostic skill, but merely a reasonable and intelligent familiarity with manipulation and a careful attention to details, in addition to precise control of environmental conditions; and it must also be capable of providing the information sought, if diagnostic ability is available.

Flour is a biological by-product. Absolute precision cannot be hoped for in any method for testing the properties of so highly complex a substance. Nevertheless, there is a type of experimental baking procedure which meets the essential conditions of a standard scientific test. At this point the writer wishes to acknowledge the assistance of E. E. Werner (1925) and R. S. Herman (1927) in supplying evidence and foundation for reasoning. Their work furnishes background. The former has practiced this procedure for nearly twenty years and the latter for approximately five years.

Their work has convinced the writer after most critical verification. Their type of procedure may be designated as the "fixed" type, in contrast to the "variable" type already discussed. That this type of test is capable of producing accurate and reliable information regarding the utility or potentialities of flour is indicated by the fact that it has done this for years, in the hands of the few who use it.

The operations concerned with the performance of this fixed type of procedure are quite simple. All flours, regardless of source, type, variety, grade, or previous history, are handled in precisely the same manner, with the same proportions and amounts of added ingredients, and are subjected to the same environmental conditions, especially degree and uniformity of temperatures, which are carefully regulated and controlled, not merely approximated.

When flour is made into a dough with water, yeast, salt, and sugar, and this dough is fermented and baked, a definite picture is presented, both in behavior during fermentation and in the final product. If another portion of the same flour is subjected to the same treatment, the picture is duplicated, within small and permissible limits of error. However, if a flour with different characteristics is subjected to the identical system of treatment, a different picture is produced, and here again the picture may be duplicated at will. Different flours treated alike produce different pictures, but individual flours always present the same picture, under this system. In a test of this type, it matters little whether or not the picture resembles a commercial loaf of bread. The point to be emphatically stressed is that each picture must have and does have a definite meaning. Whether or not one is capable of properly interpreting this meaning is another matter. If he cannot, the fault possibly lies within the individual and not with the method. The present project is concerned with the standardization of methods. It does not contemplate any attempt to standardize the experience of the operator.

From the foregoing considerations it is evident that the fixed type of method is the only one which offers any possibility of meeting the essential condition which demands the elimination of all but one variable in any standard scientific testing procedure; and is therefore the only type of experimental baking test which is worthy of serious consideration for standardization purposes. The operations are simple, so simple that almost anyone can master the technic of procedure. Granting this, it is obvious that the experimental baking test can be standardized.

Of course, the baking test must be useful as well as standard. It should serve, potentially at any rate, as a satisfactory basis for the ascertainment of the utility of the flour, and nothing is to be gained by sacrificing usefulness in order to attain standardization. In conducting experimental baking tests, operators may have slightly different viewpoints, according to the nature of their employment. No consideration of this fact can be tolerated, however, in contemplating a scientific standard procedure. It has been shown by questionnaires and discussion that the vast majority of cereal chemists are seeking the same information, in spite of the utter lack of uniformity in methods used. More than 90 per cent of experimental baking tests are intended to serve as yard-sticks with which to measure the strength or stability of flour, as reflected by its fermentation tolerance. It is chiefly from a manifestation of this property that the general or special utility of a flour may be ascertained. The ascertainment of this factor is, then, the immediate object of the experimental baking test, standard or otherwise.

Altho intelligence and experience cannot be standardized, it is appropriate to consider the manner in which the technician's reasoning and interpretative powers may even at best be expected to operate in the two types of procedure under discussion. In the first, or "variable" type, an attempt is made to bake a flour according to its own special requirements, and the value of the flour is judged by comparing the finished loaf with an arbitrary standard. That is to say, one diagnoses the flour from certain "symptoms" which may be of more or less questionable value, and then attempts to make the flour behave in accordance with the treatment which the diagnosis indicated.

If the test produces what the operator regards as an inferior loaf for his purpose, the question arises as to which is at fault, the flour or the diagnosis and treatment. This involves two kinds of reasoning process, reasoning forward from symptoms and reasoning backward from the facts. Here is an inevitable conflict, unless the operator is so expert that his diagnostic and operative skill is infallible. If such is the case, it is merely a waste of time for him to perform any sort of baking test excepting as a demonstration of his superior knowledge and skill.

In the fixed type of method, one is confronted with facts containing, at worst, but small and known factors of error. From such definite and specific facts there is the same foundation of reasoning as may be found with any chemical test. Uncertainty can be due

solely to lack of intelligence or knowledge on the part of the operator. Summarizing this phase of the discussion, it may be said that one type of procedure proposes to test the material and manufacture the product in one operation, whereas the other type merely attempts to test the material. The latter is a means to an end; the former presumes to be both the means and the end, in one.

There appears to be ample justification for the recommendation that the fixed type of procedure be tentatively adopted for testing the baking quality of flour or, at least, its fermentation tolerance.

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NOTES

"Elementary Science Course for Flour Millers," reprinted from articles appearing in *Milling* has been published by the Northern Publishing Company, 28 Paradise Street, Liverpool, England. It includes several chapters on elementary physics and chemistry, and concludes with chapters on botany and biology. A familiarity with the contents of this 60-page manual should aid millers in interpreting scientific papers covering their field of interests.